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ADDRESSING CARDIOVASCULAR RISK FACTORS AND THERAPY EFFECTS IN RHEUMATOID ARTHRITIS: IMPLICATIONS FOR ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

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To whom I love

"It is not by the gray of the hair that one knows the age of the heart."
—Edward Bulwer-Lytton

"Theory is when we know everything but nothing works.
Praxis is when everything works but we do not know why.
We always end up by combining theory with praxis:
nothing works and we do not know why."
—Albert Einstein

ABSTRACT

Rheumatoid arthritis (RA) is the prototype of chronic inflammatory disease associated with a 1.5-2 fold increased risk of cardiovascular disease (CVD) and premature mortality. Though traditional CVD risk factors are important in the pathogenesis of atherosclerosis in patients with RA, they do not fully explain the increased risk of CVD events observed in RA. This thesis aimed to explore further the mechanisms that could account for accelerated atherogenesis and CVD in RA.

In the study of established RA disease, which included patients treated during one year with TNF-α inhibitors, n=162, or the anti-CD20 agent rituximab, n=53, anti-atherogenic apolipoprotein A1 increased after commencement of therapy; this increase was not agent-specific and paralleled a reduction in RA disease activity. At the same time, apolipoprotein B and the atherogenic index did not change significantly. These favorable effects may have a potential for at least short-term improvement in cardiovascular risk in RA. On the other hand, the biologic agents caused differential effect on serum levels of anti-phosphorylcholine (anti-PC) IgM, a promising atheroprotective biomarker. Thus, these antibodies increased on TNF-α inhibitors in contrast to treatment with rituximab. The contribution of biologic agents to beneficial or harmful CVD effects needs to be elucidated in future studies.

In studies incorporating 114 participants with early RA, examined with high-resolution B-mode ultrasonography after five years of disease, the pro-atherogenic apoB and apoB/apoA1-ratio were independently associated with unfavorable carotid outcomes, while a profitable effect was associated with the anti-atherogenic, anti-inflammatory apoA1. Also, low anti-PC IgM levels longitudinally had an unfavorable association with the plaque presence at the end of observation. These results suggest that apolipoproteins and anti-PC antibodies may have independent roles in subclinical atherosclerosis in patients with RA. Further analysis, over a prolonged observation period more than 10 years, showed that the bilateral carotid plaques occurrence was associated with a subsequent CVD event. Early improvement of inflammation, pain and disability, measured as reductions in DAS28, VAS pain and the HAQ score the first year after RA diagnosis, as well as use of methotrexate were associated with a better CVD outcome. Longitudinal approach confirmed the association of low anti-PC IgM levels and, also, increasing oxidized LDL over first five years of RA disease with an adverse CVD outcome.

In a large observational early RA cohort of 741 patients followed more than 10 years, we examined the relationships of inflammatory and novel biomarkers with incident CVD morbidity and all-cause mortality. The study outcomes were tracked through the Swedish Hospital Discharge and the National Cause of Death Registries. The factors associated with adverse outcomes differed in patients with disease onset before 65 years of old and those 65 years and older. The cumulative burden of inflammation over the first two years and the presence of RA disease related autoantibodies had a value for CVD and mortality prognosis in the younger patients, while a change in inflammatory markers the first year after diagnosis had a stronger effect in the older patients. Low-dose glucocorticoids increased but use of methotrexate decreased risk of poor outcomes in the elderly. These findings imply that age stratification could add to identification of patient-at-risk, and highlight the need to treat RA early and more aggressively to improve long-term outcomes.

Taken together, these results emphasize the complexity of the associations between inflammation, anti-rheumatic therapies, atherosclerotic burden and CVD in RA. Further studies addressing indicators for cardiovascular prognosis are unmet needed.

LIST OF PUBLICATIONS

I. Ajeganova S, Fiskesund R, de Faire U, Hafström I, Frostegård J.
Effect of biological therapy on levels of atheroprotective antibodies against phosphorylcholine and apolipoproteins in rheumatoid arthritis - a one year study.
Clin Exp Rheumatol. 2011 Nov-Dec;29(6):942-50.

II. Ajeganova S, Ehrnfelt C, Alizadeh R, Rohani M, Jogestrand T, Hafström I, Frostegård J.
Longitudinal levels of apolipoproteins and antibodies against phosphorylcholine are independently associated with carotid artery atherosclerosis 5 years after rheumatoid arthritis onset - a prospective cohort study.
Rheumatology (Oxford). 2011 Oct;50(10):1785-93.

III. Ajeganova S, de Faire U, Jogestrand T, Frostegård J, Hafström I.
Carotid atherosclerosis, disease measures, oxidized low-density lipoproteins, and atheroprotective natural antibodies for cardiovascular disease in early rheumatoid arthritis -- an inception cohort study.
J Rheumatol. 2012 Jun;39(6):1146-54.

IV. Ajeganova S, Andersson ML, Frostegård J, Hafström I.
Disease related factors associated with early rheumatoid arthritis over the first two years are associated with differential predictive risk for incident cardiovascular event and mortality depending on age at onset: an observational inception cohort study over 10 years.
Manuscript.

CONTENTS

List of abbreviations

ACR	American College of Rheumatology
ACPA	Anti-citrullinated protein/peptide antibodies
AMI	Acute myocardial infarction
Anti-PC	Anti-phosphorylcholine antibodies
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
AUC	Area under the curve
BARFOT	Better Anti-Rheumatic FarmacO-Therapy
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
cIMT	Carotid intima-media thickness
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS28	Disease Activity Score of 28 joints
DMARD	Disease modifying anti-rheumatic drug
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
GC	Glucocorticoid
GEE	Generalized estimating equations
HAQ	Health Assessment Questionnaire
HDL	High-density lipoprotein
HR	Hazard ratio
IL	Interleukin
IQR	Inter-quartile range
LDL	Low-density lipoprotein
LPC	Lysophosphatidylcholine
MTX	Methotrexate
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
oxLDL	Oxidized low-density lipoporotein
PAF	Platelet activating factor
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SLE	Systemic lupus erythematosus
SMR	Standardized mortality ratio
SMC	Smooth muscle cells
TC	Total cholesterol
TG	Triglyceride
TNF-α	Tumor necrosis factor-alpha
VAS	Visual analogue scale
WBC	White blood cell

1 GENERAL INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive systemic disorder characterized by inflammation in synovial joints. Erosive arthritis resembling RA has been described in skeletons from North America dating back as far as 6,500 years ago, and in paintings RA-like features could be seen from the 15th century onwards (395). Although its name was introduced in the 1850s by Dr Augustin Jacob Landré-Beauvais (338), the first classification criteria were developed only 50 years ago (287). In industrialized countries, RA is the most common autoimmune inflammatory arthritis which affects 0.5-1.0% of adults, with 5–50 new cases annually per 100 000 adults, diagnosis based on the 1987 revised American College of Rheumatology (ACR) criteria (5). Prevalence rises with age and is highest in women older than 65 years (127).

Evidence suggests that RA develops in three phases: an asymptomatic period of genetic risk, a pre-clinical period in which RA-related antibodies can be detected, and a clinical phase with acute signs and symptoms of inflammatory arthritis (192, 222). The time duration for early RA versus established RA varies widely in the literature; while early RA usually comprises a period of weeks to months, the term "established RA" is generally used to describe patients with disease duration of 2 years or more (166). The disease has a wide spectrum of manifestations that range from mild and limited to severe and disabling. In addition to joints, extra-articular pathology may occur in up to 30% of patients, involving skin, eyes, lungs, heart and vessels (397). The natural history of RA itself includes spontaneous remission, remission induced by medical treatment or continuously progressive disease despite medication. Hence, there is a clear need to identify efficient diagnostic and prognostic indicators of the disease.

The pathogenesis of RA is not well understood. Genetic (*HLA* genes and *PTPN22*) and environmental factors likely contribute (25, 221), and autoimmune processes have been implicated, with chronic inflammation of the joint synovial membrane considered the central event in the pathophysiology. Smoking is the dominant environmental risk factor and doubles the risk of developing RA (45), and in the context of specific genes smoking may trigger RA-immune reactions to citrullinated proteins (193, 221). Other potential environmental risk factors include alcohol and coffee intake, vitamin D status, use of oral contraceptive, and low socio-economic status (85, 208).

Rheumatoid arthritis is best considered a clinical syndrome spanning several disease subsets. These different subsets entail several inflammatory cascades, which all lead towards a final common pathway in which persistent synovial inflammation and associated damage to articular cartilage and underlying bone are present. One key inflammatory cascade includes overproduction and overexpression of tumor necrosis factor-alpha (TNF–α) (111). This pathway drives both synovial inflammation and joint destruction. TNF-α overproduction has several causes, including interactions between T and B lymphocytes, synovial-like fibroblasts, and macrophages. This process leads to overproduction of many cytokines, such as interleukin-1 (IL-1), IL-6, which also drive persistent inflammation and joint destruction (229, 297). Consequences of acute and chronic inflammation in RA have a great impact on quality of life, co-morbidities such as cardiovascular disease, and mortality.

1.1 CARDIOVASCULAR MORBIDITY AND MORTALITY IN RA

In RA the mortality is higher in comparison to the general population and standardized mortality ratio (SMR) associated with RA ranges from 1.3 to 3.0 (366). The leading cause of morbidity and mortality in RA is cardiovascular disease (CVD) (355, 362) and the cardiovascular events occur approximately a decade earlier in patients with RA than in the general population (20, 91). The CVD-related morbidity in RA patients appears to be increased by two-fold or more compared to the general population (309) and is comparable to the magnitude of cardiovascular risk in type 2 diabetes mellitus (260).

Increased risks are found when analyzed by incident cardiovascular events (mostly being caused by myocardial infarctions), causes of death, or surrogate measures of atherosclerosis, such as carotid artery plaque, intima-media thickness, or coronary artery calcification. The lower survival rate in patients with RA, mainly from cardiovascular disease, has not improved over time (88). Despite milder course of RA disease over the past decades the magnitude of the overall CVD-dependent mortality is still associated with a 60% increase in risk of CVD death in RA compared with the general population (232). The magnitude of SMR and predictive factors appear to be affected by variations in study design, sample size, follow-up period, and geographic area. Established prevalent cohorts report SMR varying from 1.49 to 3.08 (126, 345, 390), greater than inception and community-based cohorts, which vary from 0.87 to 1.4 (150, 198, 215, 235, 285, 398).

Several studies have, however, shown contradictory results, demonstrating no increase in either CVD or all-cause mortality during the first 10 years of RA (198, 274), thus, no definite conclusions on the relative CVD death rate of patients with early RA can be made (184, 398). The differences seen in the cohorts may be explained, at least partly, by the changes in treatment practices with increased and early referral to secondary care, earlier use of disease modifying anti-rheumatic drugs (DMARDs), especially methotrexate, and greater emphasis on tighter disease control. The inception cohort approach itself may be responsible for some of the variations reported as it allows inclusion of a wider spectrum of RA, and is more likely to retain patients with milder disease, then, generally milder RA are also usually included in community and primary-care cohorts (375). Retrospective studies may well be biased towards the more severe patients who would be more likely to be retained in the clinic setting, partly because of co-morbidity.

Evidence that RA patients often die of CVD goes back over 50 years (279), the most comprehensive evidence comes from the recent systematic reviews of observational studies (17, 232). Although there is extensive data that patients with established RA have an increased risk of ischaemic heart disease (346), little is known about myocardial infarction and other organ specific CVD events in early RA. Essential contribution to the area has been given by the Swedish inception cohort RA study that reported the increased overall relative risk of myocardial infarction, 1.6 (95% CI 1.4, 1.9), which is present already within the first 4 years of diagnosis (164). Then, in the incident RA cohort, the risk increase of ischaemic stroke is small and non-significant, overall hazard ratio (HR) 1.11 (95% CI 0.95-1.30), but the risk is heightened after 10 or more years since RA diagnosis, HR 2.33 (95% CI 1.25-4.34) (163). Still, the relative contribution of myocardial infarction and stroke to cardiovascular mortality in patients with RA, and whether the excess of cardiovascular risk is equal in men and women as well as in seropositive and seronegative RA remain unclear.

In addition to experiencing an increased rate of CVD events, patients with RA may have a worse clinical course of an acute CVD compared with patients without RA by presenting an excess risk of fatal CVD events (204), suffering more recurrent cardiac events (91), and having less chance for survival after an acute myocardial infarction (AMI) or stroke (231, 319, 320). Atherosclerotic disease remains under-diagnosed in RA due to, at least partly, an often atypical or silent presentation which may also offer a potential explanation for the higher fatality rates associated with AMI in RA and the increases seen in post AMI complication rates (223, 386).

1.2 ATHEROSCLEROSIS

Atherosclerosis is a widespread pathologic process of medium to large arteries characterized by gradual thickness of the intima causing decreased elasticity of the vascular wall which starts in childhood and often progresses while growing older. The blood vessels most commonly affected and of clinically relevance include the aorta, coronary, carotid, cerebral and peripheral arteries. The true frequency of atherosclerosis and associated complications is difficult, if not impossible, to determine because it is a predominantly asymptomatic condition. Atherosclerosis is responsible for CVD events when a number of lipid regulatory and inflammatory mechanisms within the arterial wall is disrupted leading to blood clotting and flow restriction to target organ.

Atherosclerosis and CVD are believed to be attributed to traditional risk factors, such as family history, cigarette smoking, high blood pressure, hypercholesterolemia, diabetes mellitus, obesity, estrogen replacement therapy (218), as well as additional factors such as age, male gender, race, physical inactivity, the individual response to stress, excessive alcohol consumption, and low high-density lipoprotein (HDL) cholesterol levels (8, 252, 400).

1.2.1 Pathogenesis of atherosclerosis

In short, initiation of atherosclerosis starts with an increased permeability of the dysfunctional arterial endothelium, which facilitates migration of cholesterol-filled low-density lipoprotein (LDL) particles into the vessel wall. After migration into the intimal layer, LDL particles undergo modification and oxidation, inducing the endothelial cells to express leukocyte adhesion molecules and initiating an inflammatory response in the artery wall with attraction of monocytes to the lesion (315). Once in the sub-endothelial space, monocytes are transformed to macrophages, and subsequent incorporation of oxidized LDL via endocytosis by scavenger receptors differentiates them further into foam cells. Foam cells eventually precipitate in the vessel wall causing fatty streaks, the earliest recognizable lesion of atherosclerosis, which further stimulates the inflammatory process. Further attraction of macrophages is promoted, together with migration of proliferating smooth muscle cells (SMC) from the medial into the intimal layer of the arterial wall. SMCs produce collagen, which results in formation of a fibrous cap overlying the atheroma and covering the atherosclerotic plaque (213, 214).

Recent data support the assumption that atherosclerosis is an inflammatory autoimmune disease (160, 288, 309). In RA, IL-1, tumor necrosis factor-alpha (TNF–α), and other inflammatory cytokines produced in the joints, spill into the circulation, where they can up-regulate adhesion molecules and other pro-inflammatory ligands leading to leukocyte chemotaxis into vessel walls (43). C-reactive protein (CRP), which increases in periods of high rheumatoid inflammatory disease activity,

may also have a pro-atherogenic role as it stimulates macrophages to produce tissue factor, an important pro-coagulant found in atherosclerotic plaques. Similar cellular immune system abnormalities, as perturbation of the T-cell repertoire with the emergence of CD4(+)CD28(null) T-cells, have been described in RA and unstable angina (216). Thus, immune-pathogenic mechanisms may link atherosclerosis to joint damage in RA, and the inflammatory process in atheromatous plaques may bear some resemblance to synovial inflammation of RA.

There are two different approaches explaining the interplay between RA and CVD. On the one hand, RA plays an important part in modulating the risk of CVD events, which accumulates during the RA disease course due to RA-specific factors such as immune dysregulation, systemic inflammation or treatment with oral glucocorticoids or non-steroidal anti-inflammatory drugs (NSAIDs) (184). On the other hand, RA and CVD share risk factors such as genes, smoking and physical inactivity, and also, traditional and RA disease related CVD risk factors seem to act synergistically in accelerating atherosclerosis in early RA (79). There is evidence concordant with both of these theories, suggesting a combination of different modes of interaction (24, 108, 350).

Inflammation is important in the early vascular lesion that initiates plaque formation and growth, and also plays a role in the plaque ulceration that triggers thrombosis, thus, elevations in CRP and other inflammatory markers could be secondary to endothelial inflammation. It is also possible that inflammatory processes arising outside the vascular endothelium could act remotely to promote atherosclerosis (78). Underlining the pathogenic importance, CRP is proposed as both a marker and a mediator of cardiovascular disease (211, 284). However, since inflammatory activity in apparently healthy individuals is probably related to inflamed atherosclerotic vessels or established CVD risk factors, the suppression of inflammation in these patients might not be equivalent to suppressing disease activity in RA. Nonetheless, successful suppression of RA disease activity through anti-rheumatic treatments may change conventional risk factors and reduce CVD risk in RA (237, 294, 307, 335).

Of note, etiology is the most difficult issue to understand in autoimmunity as chronic inflammation is associated with most, if not all, of traditional CVD risk factors, and the interplay of various factors is complex, then, it remains unclear whether inflammation *per se* is the cause or the effect of other pathogenic processes. Also, the pathogenesis of atherosclerosis associated with autoimmune disease may differ in various inflammatory phenotype and genotype settings. It is possible, that a different set of risk factors contribute to early-onset CVD in RA compared to late-onset CVD, as well as in early compared to established RA disease.

1.2.2 Risk factors of atherosclerosis in RA

Growing evidence shows that atherosclerosis cannot be fully explained by conventional cardiovascular factors alone (159), and CVD morbidity and mortality in RA occur at rates greater than would be expected from the profile of established CVD risk factors (83). The Framingham risk score are thought to account overall for 50% of CVD events in general population (281). As to patients with RA, commonly used prediction models are insufficient for the estimation of CVD risk. Thus, the Framingham risk score can substantially underestimate CVD risk in patients with RA in both genders, especially in older ages, and in patients with positive rheumatoid factor or persistently elevated erythrocyte sedimentation rates (ESR); and the

Reynolds risk score, which include CRP, has similar deficits in CVD risk prediction (63).

Also, traditional risk factors seem to have different impact on risk for CVD events in RA and non-RA subjects. Age and male gender are the strongest contributors to the extent of atherosclerosis in the general population (151) and in RA (79). Age has a similar impact on CVD risk in patients with RA compared with non-RA subjects, but male gender and personal cardiac history appears to have a weaker association with CVD events in RA (145). The effect of smoking on CVD in the population is overwhelmingly evident, and this effect interacts synergistically with other CVD risk factors as age, gender, hypertension and diabetes (151). The effect of smoking (vs. non-smoking) is less in RA patients compared to control subjects, hazard ratios for CVD 1.3 and 2.2 respectively, but still, smoking is an important and modifiable augmenting factor for subclinical atherosclerosis in RA (136, 145). Smoking history is common in RA, and whilst it does not seem to confer the same relative risk of CVD events when compared to the general population, cigarette smoking certainly associates with more severe RA and also, with RF-positive or ACPA-positive status; both these factors independently associate with higher subclinical atherosclerosis and CVD mortality (135, 148, 219). In addition, dyslipidemia, hypertension, metabolic syndrome and obesity are highly prevalent in RA affecting about 60%, 70%, 40%, and 25% of patients, respectively (66, 84, 180, 253, 334).

1.2.3 Carotid atherosclerosis in RA

A diagnostic tool to detect premature atherosclerosis is carotid ultrasound, which is a non-invasive, simple, widely available, relatively inexpensive method assessing structural changes in the arterial wall. Carotid intima-media thickness (cIMT) of the common carotid artery, determined by ultrasound, is a useful surrogate indicator of an early subclinical stage of macro-vascular atherosclerosis disease (313). cIMT is also a strong indicator of future CVD events in otherwise healthy individuals and can improve coronary risk prediction beyond traditional risk factors (220, 242), particularly in subjects with low-grade inflammation as assessed by CRP levels (44). It is known that ultrasound and histological images of the cIMT are highly correlated (257). Moreover, carotid and coronary atherosclerosis are highly correlated as well (399), but this correlation does not imply that carotid cIMT is a predictor of the severity and extent of coronary atherosclerosis. However, carotid cIMT may perform as a marker of atherosclerosis in other vascular beds (74), and cIMT mean $\geq$ 1 mm is considered to be a reliable indicator of generalized atherosclerosis associated with increased risk of coronary heart disease in asymptomatic individuals, independent of major CVD risk factors (47). A systematic review and meta-analysis has shown that for an absolute cIMT difference of 0.1 mm the future risk of AMI increases by 10% to 15%, and the stroke risk increases by 13% to 18% (220).

However, the accuracy of cIMT as a marker of atherosclerosis has been questioned by the fact that main predictors of medial hypertrophy or intimal thickening of the common carotid artery are age and hypertension, which do not necessarily reflect the atherosclerotic process, and normal cIMT values should be defined on the basis of age, sex, ethnicity, body size, and muscularity (336). Studies of the pathology indicate that cIMT mainly represents hypertensive medial hypertrophy or thickening of smooth muscles in the media, whereas atherosclerosis is largely an intimal process. Age-related thickening of intimal and medial layers of the common carotid also occurs in the absence of overt atherosclerosis (115). In contrast, carotid plaques probably represent a later stage of atherogenesis related to inflammation, endothelial

dysfunction, oxidative stress, and smooth muscle cell proliferation. Thus, it has been hypothesized that carotid plaque is a distinctive phenotype of atherosclerosis, not a simple continuum of cIMT progression (332). The review of prospective epidemiological data in the general population has come to conclusions that cIMT is an independent but relatively modest (as judged by absolute risk) predictor of coronary heart diseases (CHD). Then, ultrasonography-assessed carotid plaque is superior to cIMT for CHD prediction (168, 312).

The accelerated and premature burden of atherosclerosis in patients with rheumatic diseases has been reported (80, 366), but the evidence is still not definitive (191) as most of the performed studies have been cross-sectional in design and with small sample sizes. It has been questioned whether the studies did indeed provide evidence for accelerated atherosclerosis in RA or reflected alternative processes, where in conditions of high-grade inflammation increases in carotid measures simply reflects current, potentially reversible inflammation of the vessel wall rather than more permanent structural vessel changes. Thus, a single measurement of cIMT in this context could not be a good predictor of future CVD events (378).

Nevertheless, cIMT has been found to be increased already as early as within 12 months of symptom onset of RA (158). In a recent meta-analysis it was demonstrated that cIMT is significantly increased in patients with rheumatic diseases, an overall mean difference of 0.055 mm (95% CI, 0.048-0.063) compared with healthy controls adjusted for age, sex and disease duration, which can be estimated to be a 7- to 8-year increase in age, with pre-existing CVD excluded (358). As inflammation is present even in the quiescent stages of RA disease, a cIMT increase per unit of age in proportion to RA duration has been estimated as 0.154 mm/10 years among patients with RA for not more than 7 years, to 0.295 mm/10 years among patients with RA for at least 20 years (80). When considering the presence of plaque, a significant difference between patients and controls has been also reported (358).

Whether the chronic inflammatory milieu in RA disease may confound ultrasonography findings is unclear to date. Nevertheless, it has been supported that cIMT measures may be used as a predictor of CVD regardless of the absence of classic cardiovascular risk factors, and subjects with cIMT >0.91 mm had a high risk of suffering CVD events in the following 5 years, while those with cIMT <0.77 mm had none CVD (142). Also, it has been shown that carotid plaque presence was predictive for acute coronary syndrome in RA (104). Still, the meta-analysis of carotid measures in heterogeneous rheumatic populations has not shown agreement between cIMT and carotid plaque measurements (358). Taking into account differences in the amount and type of inflammation in rheumatic diseases, detection of carotid plaques by ultrasonography, not cIMT, may be a more reliable predictor of CVD events in patients with RA, and plaques could probably be expected potentially more inflammatory active, and thus, rupture-prone (292).

Few reports are available addressing association between RA disease characteristics (mainly disease duration) and carotid atherosclerosis (77, 137, 143, 178, 199, 361), and fewer have addressed progression of carotid atherosclerosis (11, 113, 137, 141, 318, 401), and further association of carotid atherosclerosis with future CVD events in early RA (104). While it could be logically presumed that anti-rheumatic treatments may affect carotid atherosclerosis, reports have yielded mixed results, and data are yet limited to biologic agents, primarily TNF-α blockers and rituximab (11, 77, 113, 137, 141, 182, 183, 310, 391), methotrexate (113, 134, 361), and glucocorticoids (81, 104, 134, 137, 155, 199, 401).

1.2.4 Clinical atherosclerotic complications

Etiologies of acute atherosclerotic complications have been defined as plaque rupture, plaque erosion and calcified nodules; and ulcerative changes, cap disruption and intra-plaque hemorrhage are preludes to the luminal thrombosis (384). Lipid accumulation, apoptosis, proteolysis, thrombosis and angiogenesis have been shown to be involved in the progression of the atherosclerotic lesions and plaque vulnerability (157). It must be emphasized that the precise mechanisms behind plaque progression are not understood, and that not all vulnerable plaques are likely to progress to rupture (195), which limits the positive predictive value of plaque imaging for CVD events and identifying the patient at risk.

Morphologically, intact thin fibrous cap less infiltrated by macrophages, smaller necrotic core size, positive remodeling/healing, and less degree of calcification are features of stable plaques. Repeated intra-plaque hemorrhage, necrotic core expansion, changes in lipid composition of plaques, hypoxic environment created by increased lesion burden and inflammatory macrophages, defective clearance of apoptotic cells, decreased collagen fiber production, few smooth muscle cells and weakened thinner fibrous cap, angiogenesis, adventitial inflammation, and outward remodelling may precipitate plaque rupture (116, 125).

In an autopsy study of cases of sudden coronary death, 55% to 60% of the subjects had underlying plaque rupture as the etiology, whereas for 30% to 35% - erosion, and for 2% to 7% thrombi were attributed to calcified nodules (116). Interestingly, up to 75% of the cases of acute myocardial infarction (AMI) may be ascribed to plaque rupture, at the same time approximately 37% of the women with AMI had plaque erosion but only 18% of the men (13). Overall, the etiology of luminal thrombus is dependent on age and sex, where plaque rupture is a dominant mechanism in men regardless of age and in postmenopausal women older than 50 years, then, plaque erosion appears to be the primary cause of thrombus in women aged <50 years (38).

Furthermore, arterial calcification, a regulated process similar to bone formation, frequently coexists with atherosclerosis, and approximately 15% of the carotid artery plaques contain calcifications (167). However, histopathology studies indicate that patients with extensive calcification of the carotid plaques are less likely to have symptomatic CVD disease, thus, it has been suggested that plaques calcification may be a plaque-stabilizing factor and protective (167). In contrast, the vulnerable plaques tend to be uncalcified or “mixed”.

Vulnerable plaques tend to occur at multiple sites, and several authors have advocated the concept of the vulnerable patient, defined by high atherosclerotic burden, vulnerable plaques, or pro-thrombotic coagulation state (116). Thus, detection of rupture-prone vulnerable plaques is crucial in prevention. Modern vascular imaging, such as fluorescence imaging, MRI, CT-angiography, optical coherence tomography etc., improves definition of high-risk lesion in the clinic, yet, no existing diagnostic modality can certainly identify these lesions. To date, specific treatment approaches targeting unstable lesions are not known.

Despite the clinical relevance of the mechanisms of progression of artery lesion in atherosclerosis and great interest for plaque stability in modern cardiology, as aforementioned, research addressing this question in rheumatic diseases is in its cradle. It seems possible that the mechanisms of atherogenesis, plaque formation, plaque rupture and atherothrombosis differ in RA and non-RA populations. Local and systemic inflammation are integral parts of plaque advancing and destabilization, however, in non-rheumatic individuals inflammation constitutes only 2% to 5% of total

lesion volume (106), whereas in rheumatic disorder the contribution of inflammation may be expected more significant. This is supported in an autopsy study with histological evaluation of coronary arteries, where patients with RA compared to non-RA controls had less histological evidence of atherosclerosis but greater evidence of inflammation, more vulnerable and inflamed high-risk plaques with similar trends for subjects with heart failure (16). Overall, no significant difference in grade of stenosis or number of acute coronary lesions was found, but among subjects with CVD, 54% of non-RA controls had grade 3-4 lesions in left main artery versus only 7% of patients with RA (16). Then, in analysis of atheromatous plaques in RA patients without overt CVD, disease activity was associated with carotid plaque vulnerability, not cIMT measurement or plaque presence *per se* (333).

1.3 LIPIDS IN ATHEROSCLEROSIS AND RA

It has been postulated that among traditional risk factors disorders in lipid metabolism are central to the development of atherosclerosis, which were described already in 1976 by Ross (289). The progress of atherosclerosis is a complex process, in which bi-directional interaction between lipids and inflammation is fundamental to all its stages (210). Healthy endothelium exerts a number of vasoprotective effects such as vasodilation, suppression of smooth muscle cell growth and inhibition of inflammatory responses, thereby helping to protect against atherosclerosis. Multiple lipid abnormalities, likewise systemic inflammation, may disrupt endothelial homeostasis.

As dyslipidemia has been found already prior to a diagnosis of RA (239), the concept of dyslipidemia as an etiological factor for RA has been proposed (367). Dyslipidemia may be present in both early and advanced RA disease (251). In early untreated active RA, the lipid profile is characterized by mild dyslipidemia with low total cholesterol (TC), low high-density lipoprotein (HDL) and low triglycerides (TG). At first glance this appears to produce a less atherogenic profile, however, HDL levels fall disproportionately more compared to TC levels resulting in an increased atherogenic index (TC/HDL ratio) (59). In addition, HDL function is abnormal in RA, because this molecule is unable to protect LDL from oxidation; such altered HDL has been reported to associate also with active disease (49).

The studies on pattern of dyslipidemia in RA are though not unanimous in conclusions. Some studies support a typical pro-atherogenic lipid profile in early RA, with higher serum levels of TC, LDL cholesterol and TG but lower serum HDL cholesterol, as well as smaller LDL particle size compared with controls (133, 286). In other studies, patients with early or advanced active RA have been characterized by relatively low concentrations of both TC and HDL cholesterol (33, 278).

Lipid abnormalities seem to be linked to the systemic inflammation, at least partly mediated by inflammatory cytokines, such as TNF-α, with potential for a feed-back loop. Thus, cytokines lead to dyslipidemia that promotes oxidation, which in turn mediates further cytokine release at endothelial cells (110). Also, dyslipidemia in RA may be influenced by an array of other factors including genetic predisposition (351), gender, menopausal status, disease activity (55, 396), RF-positivity (55), reduced physical activity (99), and drug therapy (351).

As aforementioned, associations of lipids with CVD in RA may be confounded by inflammation; further, lipids may have paradoxical associations with the risk of CVD in RA, whereby lower TC and LDL levels, and even lower atherogenic ratios (TC/HDL and LDL/HDL ratios) are still associated with increased cardiovascular risk (240).

Albeit the superiority of apolipoproteins in predicting CVD remains an area of debate, the mounting evidence addresses the importance of apolipoproteins as powerful lipid-related [e.g., apolipoprotein A1 (apoA1) with HDL, and apolipoprotein B (apoB) with LDL] indicators of CVD risk and important treatment targets in the general population (363-365). It has been postulated that apoA1 possesses both anti-inflammatory and anti-oxidative effects. In conformity with this hypothesis, infusions of apoA1 or HDL to humans and animals resulted in reduced inflammation and atherosclerosis (249, 273). Besides, it has been shown that apoA1, in combination with lipid-lowering drugs, significantly reduced clinical disease activity and progress of erosions, as well as improved the anti-inflammatory properties of HDL in collagen-induced arthritis, thus demonstrating a dual therapeutic potential in autoimmune diseases (48). In patients with RA apoA1 and apoB correlate significantly with HDL-cholesterol and TC, respectively (278). ApoA1 levels decrease in active RA (55, 134, 256, 396), mirroring the suppression in HDL. Also apoB levels have also been reported to be reduced at high levels of inflammation (130), but to a lesser degree than apoA1 (130, 256, 396).

The use of lipid ratios (TC/HDL and LDL/HDL) and apolipoprotein ratio (apoB/apoA1 ratio) is gaining popularity as the ratios have been shown to confer a greater predictive value of first CVD event and probably a better target for lipid-lowering therapy than individual lipid levels in the general population (112, 363). Still, it is not proved if a ratio change caused by an increase in apoA1 is equivalent to that caused by a reduction in apoB. All three lipid ratios have been reported to increase in active RA (93, 256, 396). To use lipid ratios has been considered attractive for risk stratification in RA as they may overcome effects of inflammation on individual lipid levels (59, 262, 349), however, other authors could not confirm this conclusion (240).

In RA, dyslipidaemia combined with enhanced activity of pro-inflammatory cytokines in chronic inflammatory milieu lead to a pro-oxidative state (177), which further promote oxidative modification of LDL to the highly atherogenic oxidized LDL (oxLDL), both within the synovial fluid and the plasma of RA patients (173). oxLDL is likely to be of great importance in atherosclerosis and constitutes a major part of early vascular atherosclerotic lesions. It exhibits enhanced pro-atherogenic properties, through its ability to more readily infiltrate the arterial wall, to form foam cells and to initiate a localized inflammatory response (293). Biological actions and consequences of oxLDL include activation of monocytes, endothelial cells, T-cells and B-cells (29, 124), injuring endothelial cells, expressing adhesion molecules, recruiting leukocytes and retaining them (174). Further, oxLDL contains other oxidized products, including platelet activating factor (PAF) -like lipids (123, 124), and its pro-inflammatory, immunogenic and pro-thrombotic properties are probably mediated through the PAF-receptor, to which phosphorylcholine (PC) is the major ligand (12). Also, oxLDL triggers the CD40/CD40L signaling pathway through LOX-1, an oxidized endothelial receptor, which might also lead to a pro-inflammatory reaction and induce endothelial injury (205).

In the general population, increasing plasma oxLDL levels have been associated with a gradually increasing risk for CVD events (170, 343), and high titers of oxLDL have been detected in patients with AMI (96, 353). Further, the plasma oxLDL level has been described as the strongest predictor of CVD events compared with a conventional lipid profile and other traditional risk factors (230). It has been suggested that elevated oxLDL can play a role in the transformation from stable to vulnerable unstable plaque (186). Also, increased levels of antibodies against oxLDL have been found in patients with an early-onset peripheral vascular disease (27).

In RA, studies investigating the possible contribution of elevated oxLDL or antibodies against oxLDL to carotid atherosclerosis and CVD outcomes are limited, and the results are contradictory (65, 114, 259, 308, 359, 392). However, there are reports showing that serum oxLDL and antibodies against oxLDL are raised and correlate with disease activity, independently of other inflammatory markers, suggesting the importance of oxLDL in a chronic inflammatory condition *per se* (65, 187, 259, 393). Further studies are warranted to determine the relationship between oxLDL and inflammation, and the potential predictive role of oxLDL for accelerated atherosclerosis and CVD in RA.

Anti-rheumatic medications alter the lipid profile with the most widely reported changes, at least short-term, being elevation in HDL or apoA1 levels, but also, increase in TC and LDL levels, without an overall change in the atherogenic indexes. The most likely mechanism to account for such changes in the lipid profile is suppression of inflammation potentially reflecting a reversal of the previously inflammatory-mediated suppression of the lipid profile. Interestingly, some medications have been shown to exert drug specific effects on the lipid profile, for example, hydroxychloroquine has been reported to produce a less atherogenic lipid profile by lowering TC, LDL and TG, and increasing HDL levels (237, 238, 282), while a pro-atherogenic pattern has been demonstrated in the studies evaluating treatment with tocilizumab (103, 132, 175, 181, 246, 247, 317). However, for other agents there are contradictory evidences on the impact of the drugs on lipid levels. Thus, for methotrexate both improved, mostly unchanged and impaired lipid profile have been reported (133, 255, 282, 291), as for glucocorticoids (33, 131, 133, 155, 255, 282), TNF-α blockade (7, 35, 64, 68, 69, 129, 188, 261, 268-271, 291, 295, 328, 331, 347, 373, 382, 385), and rituximab (144, 182). Generally, the conclusions of the studies are limited by small sample sizes, diversity in patient selection, different approaches used to adjust for disease activity, cross-sectional or non-randomized design (with exception for several studies of tocilizumab), various dosage of drugs used, different observation periods and concomitant use of other DMARDs.

The understanding of dyslipidemia in RA remains far from complete. An overall net effect of suppressing inflammation may be more important for CVD risk reduction than the effects on lipid profiles. Still, improving RA disease *per se* does not seem enough to treat the accompanying dyslipidemias, and in CVD prevention dyslipidemia in RA should be managed as in the general population (258). Interestingly, hitherto, comparable lipid-lowering effect has been reported in patients with RA and those without RA, despite lower baseline cholesterol levels in RA patients (301, 303), and also, discontinuation of statin therapy in patients with RA for more than 3 months has been shown to associate with increased risk of myocardial infarction (76).

Numerous challenges remain to establish the relative contribution of dyslipidemia to CVD in RA. Further studies are required to investigate the changes in lipid structure and function, the mechanisms behind beneficial or adverse effects of drugs on lipid profile, and more intricate effects of drugs on lipids, such as lipid subfractions, lipid modifications, LDL oxidation, and the impact of these changes, probably drug-specific, on CVD risk.

1.4 NATURAL ANTIBODIES IN ATHEROSCLEROSIS AND INFLAMMATION

The existence of B-cells subsets has not been clearly established in humans. In mice, distinct B-cell types have been described, B1-cells, producers of natural antibodies, B2-cells, which require T-cell interaction for activation and production of antibodies, and B10-cells, which are negative regulators of inflammatory immune response. B1-cells spontaneously and continuously secrete antibodies, reacting with microbial polysaccharide and lipids, and it is possible that microbial flora in the gut are the source of antigens that stimulate production of these antibodies in the peritoneum. These self-binding, highly cross-reactive, low-affinity, non-mutated antibodies make up a considerable amount of the circulating IgM in humans and are referred to as natural or background antibodies because they occur at low abundance independently of antigen stimulation, and provide a non-adaptive T-cell independent first line of defence against pathogens (32, 92). The natural IgM antibodies can be switched to IgG, without B-cell antigen receptor stimulation, in response to pathogen-associated molecular factors such as bacterial wall components. At birth, humans already have substantial levels of circulating IgM antibodies, which reflect a functional neonatal B-cell compartment ready to contribute to neonatal host defense (311).

Phosphorylcholine (PC) is a component of phospolipids and exists in LDL cholesterol and plasma membranes. The specificity of the protective autoantibodies is skewed towards phospholipid moieties. Natural antibodies against PC, anti-PC, can react to PC on bacteria, oxLDL and apoptotic cells, but not to those on unoxidized phospholipids, native LDL and viable cells, thus, they have a role in maintaining the homeostasis of the immune system (305). Most humans have a substantial immune response to PC and natural PC-specific antibodies (anti-PC) have been reported to constitute between 5-10% of the total IgM pool (248). Natural IgM antibodies possess a high overall binding avidity, a feature that makes these antibodies particularly effective in binding antigens with a repetitive structure on the surface of cells, tissues, bacteria and viruses. Since they also recognize a variety of self-antigens, they bind to a number of self or foreign antigens, e.g. nucleic acids, phospholipids, erythrocytes, serum proteins, and cellular components, and thus, anti-PC serve in the clearance of pro-inflammatory agents, apoptotic cells and released auto-antigens exposed during stress, tissue damage and inflammation (101). Thus, it has been suggested that anti-PC IgM has a housekeeping role of a clearance system for aging and/or oxidized or otherwise modified lipoproteins and dying cells (58); if so, a high level of anti-PC is beneficial, while a low level may predispose for chronic autoimmune inflammation and atherosclerosis (306). The beneficial effects of anti-PC antibodies have been demonstrated both *in vitro* (53, 57, 73, 306), and *in vivo* (31, 39, 107).

Many of the biological effects of oxLDL are exerted through platelet activating factor (PAF)-like lipids and lysophosphatidylcholine (LPC) (227, 341). Both agents are generated in the oxidation of the omnipresent phospholipid, phosphatidylcholine, which is abundant in LDL and plasma membranes (56, 226). This group of pro-inflammatory/cytotoxic compounds generated in the oxidation of LDL exhibits the PC epitope, and PC is one of the key epitopes found on oxLDL but not native LDL (28, 306). Anti-PC extracted from human serum has been demonstrated to inhibit the pro-inflammatory effect of PAF, a potent phospholipid activator involved in changes to vascular permeability, lipid oxidation, chemotaxis of leukocytes, and believed to be major inflammatory mediators in the atherosclerotic plaque (340). Further, anti-PC may inhibit uptake of pro-atherogenic oxLDL in macrophages by scavenger receptors

such as CD36, thus, anti-PC may prevent the formation of foam cells (73) and clearance of atherosclerotic plaques (36). Additionally, anti-PC has been shown to inhibit L-α-LPC-induced death of immune cells (119), and considering the richness of LPC in plaques, this mechanism may be important in plaque stabilization.

Several recent studies have indicated that deficiency of anti-PC of IgM subclass could play a role in atherogenesis and chronic inflammation. Low levels of anti-PC have independently predicted CVD in general; moreover, a negative association between anti-PC levels and the development of human atherosclerosis was proposed (122). Further, low levels of anti-PC associate with the development of ischemic stroke and increased risk for AMI, and could be an indicator for subsequent CVD, particularly in men (73, 118, 154, 314). In contrast, high anti-PC levels may be protective and associate with reduced rate of atherosclerosis progression, independent of known CVD risk factors (119, 339).

Regarding properties of anti-PC in chronic autoimmune condition, two cross-sectional studies in systemic lupus erythematosus (SLE), have demonstrated association of low anti-PC IgM levels with both carotid plaque occurrence and disease activity, then, low levels of anti-PC IgM were more common in SLE patients than in age- and sex-matched population-based controls (9, 340). The knowledge about levels and effects of anti-PC in autoimmune diseases and theirs value for CVD prediction is otherwise limited.

The understanding of the anti-PC phenomenon remains a challenge for the future. It is particularly important to continue the search for novel therapeutic concepts blocking disease-promoting immune processes. Modulation of the immune response using vaccines against PC represents a potential therapeutic strategy in the management of atherosclerotic disease. There is a clear need for the development of innovative and sensitive diagnostic approaches, and anti-PC may be an interesting such an indicator of deficiency of immune state and a prognostic marker of efficacy of anti-inflammatory drugs. Combining mechanistic findings made in the experimental systems with evaluation of biomarkers of pathological reactions of innate immune systems deserve further evaluation in larger cohorts to make it possible to achieve the aims of predictive, preventive and personalized medicine.

1.5 RA DISEASE FACTORS AND CARDIOVASCULAR OUTCOMES

In RA, the balance between pro- and anti-inflammatory cytokines is tilted toward continued inflammation. The normally low levels of pro-inflammatory cytokines become chronically increased both in serum and synovial fluid causing prolonged inflammation (337). Evidence suggests that the sooner the RA patients are treated, the better is prognosis. The availability of markers that could help to identify patients with more aggressive, rapidly progressive RA with poorer prognosis would offer a rational basis for early and aggressive treatment. In that way it may be possible to avoid many irreversible clinical complications.

For many years, clinical researchers have collected observational data on small and large patient cohorts in order to identify those characteristics that best predict clinical outcome. These studies are highly diverse in almost every respect, such as design (for example, retrospective as opposed to prospective), the definition of the status of the disease, disease duration, number of patients included, length of follow-up, definitions of outcome variables, means to assess outcomes, baseline characteristics of the patients (for example, ethnicity, age, sex, smoking habits or social status), medication allowed before and during the study period, management of the patients

and intensity of intervention (316). This diversity makes a broad comparison of the results of various studies very difficult, if not impossible. Nevertheless, from the findings of many studies, several biological markers indicative of active inflammation seem to be dependable surrogate markers of worse clinical outcomes.

Here, it will be discussed key questions of some serological, immunological, clinical and functional markers in association with risk for CVD and mortality in the context of RA. The predictive value of all of these markers has been challenged in several studies, and none has been established as the single most reliable CVD prognostic factor in clinical practice. It should be noticed that in relation to a comprehensive research on CVD and mortality outcome in RA, reports on predictive value of inflammatory markers in this context are sparse.

1.5.1 Markers of inflammatory activity

Erythrocyte sedimentation rate (ESR) together with C-reactive protein (CRP) is the most frequently used laboratory measure reflecting disease activity. CRP is the most extensively studied systemic marker of inflammation, an acute-phase protein featuring a homopentameric structure and Ca^{+2}-binding specificity for phosphocholine. CRP has an important immune-modulatory role, either activating or inhibiting the inflammatory responses depending on the biological context, such as activation of complement, anti-tumor effect, binding of LDL, modulation of superoxide and nitric oxide release, binding to phagocytic cells, blockade of activation of macrophages, increased synthesis of IL-1R antagonist, inhibition of leukocyte adhesion to endothelial cells, pro-coagulation and opsonization of bacterial cell fragments (86). Most functions of CRP are easily understood in the context of the body's defenses against infective agents. However, at the moment, neither the physiological functions of human CRP nor its possible role in disease is well known (86). CRP is considered to be a more specific biomarker for RA disease activity than ESR, since the hepatic production of CRP reflects the effects of inflammatory cytokines IL-1, IL-6 and IL-17 in the liver, although extra-hepatic production can also contribute to systemic concentrations (98). Interestingly, CRP reflects more short-term changes in disease activity compared with ESR, which reflects disease activity of the previous weeks (371), thus, the timing of measurement may have important implications for the results of the studies.

In an otherwise healthy population, elevated CRP levels are associated, though relatively modestly, with an increased risk of CVD events (71, 283). The biologic explanation of this association is likely due to chronic low-level inflammation in the vascular intima. Although elevated CRP is present in atherosclerosis it seems not directly responsible for it, as it is also associated with other factors which are themselves coupled with CVD and mortality risk, e.g., socioeconomic and lifestyle factors, such as smoking and BMI, and the *CRP* genotype (86). Thus, elevated CRP (383), as also ESR and other inflammatory variables, is likely a nonspecific indicator of more general illness and, thus, CVD, but not a specific indicator of vascular disease.

The question of the comparative usefulness of the ESR and CRP in the assessment of RA activity has been addressed in the study by Wolfe *et al*. in which complete rheumatologic examinations and laboratory tests were assessed (visual analog scale pain and global severity, joint count, functional disability, depression, a composite measure of disease activity, ESR, CRP, hemoglobin, RF, immunoglobulins, haptoglobin, alpha 1-antitrypsin and albumin). The average correlation with the

clinical variables was similar for ESR and CRP, but a substantial portion of the correlation with ESR was explained by the immunoglobulins, RF, and hemoglobin rather than the acute phase response. Still, because ESR is sensitive to immunoglobulins and RF, it may measure general severity better than CRP, even though it is a poorer measure of inflammation (387). These data, thus, have implied that the combination of ESR and CRP yields useful information that is often not apparent when only a single test is used.

In patients with RA, CRP and/or ESR can predict atherosclerosis measured by cIMT and/or the presence of carotid plaque (82, 140, 158, 254), but in other studies this association was not confirmed (80, 104, 176, 199). Then, women with RA and SLE, without prior history of diabetes or CVD, have been reported to have a significantly increased odds of having coronary artery calcium (CAC) and more extensive CAC, detected by electron-beam computed tomography, compared with matched healthy controls, which was explained, at least partly, by differences in CRP levels (179).

The association of inflammatory indicators in RA with risk of CVD morbidity and mortality has been explored in several studies. Both baseline ESR in early as well as established RA (34, 169, 390, 398) and elevated CRP $\geq$ 5 mg/l in newly onset inflammatory polyarthritis (149) have been shown to independently predict CVD death or all-cause mortality; on the contrary, other studies could not demonstrate this association (260, 290). In a study of RF-positive RA patients, a high last registered ESR before event was strongly predictive of incident CVD (360). It has also been found that the risk of CVD death was significantly higher among patients with early RA and a ESR values of $\geq$ 60 mm/h on $\geq$ 3 occasions, hazard ratio (HR) 2.03 (95% CI, 1.45-2.83), controlled for traditional cardiovascular risk factors and co-morbidities (224). Then, heart failure is preceded by an inflammatory activation as shown by ESR in early RA patients, free of heart failure prior to RA incidence date (225). Also, in established RA disease, approximately similar associations between the mean ESR or CRP levels and an increased risk for CVD events and CVD mortality have been found, underlying the importance of a chronically high inflammatory response for development of CVD in RA (139). Here again, it would be important to mention the synergistic action between inflammatory and traditional CVD risk factors, thus, in the recent inception RA cohort study the risk of a new CVD was potentiated by the combination of ESR at baseline and traditional factors (169).

1.5.2 Serological markers

RF (rheumatoid factor) and ACPA antibodies (anti-citrullinated protein/peptide antibodies) are established biomarkers used in both diagnosis and prognosis of RA, and their presence predicts a more aggressive, destructive disease course (117, 299, 300). In contrast to the inflammatory variables outlined above, RF and ACPA are mostly stable over time, thus, these markers can be regarded as characteristic of a particular individual's disease.

RF is one of the auto-reactive natural polyclonal antibodies of predominantly IgM isotype reacting with the Fc portion of IgG, whose primary role is believed to be the first line defence against infection. Like other natural auto-reactive antibodies the main source of RF is considered to be the B1-cells. In healthy state, low-affinity polyreactive IgM RFs are probably beneficial as they help in clearance of the formed immune complexes (46). The "natural" low-affinity RF is produced by CD5(+) B-cells found in healthy individuals during the course of a physiological response to

various viral and bacterial infections and during certain inflammatory conditions, malignancies and organ transplantation (2). The presence of RF may be useful and beneficial early in the course of a bacterial or viral infection. Interestingly, RF levels are higher in secondary compared with primary infections, and even higher in the sera of latent infected individuals suggesting that individuals with higher RF may not be clearing infections (244).

In RA, RF-producing B-cells are stimulated and continuously produce RF. Such RF is mono-reactive, somatically mutated and has increased affinity; also, it may be produced by B-cells within the synovium. Large amounts of high-affinity RFs may be harmful by participation in a vicious cycle of autoantibody production by stimulation of self-lymphocytes, and/or deposition in blood vessels. The production of RF can be taken as indicator of severe RA disease with a striking involvement of B-cell activation (89). RF can be detected in 60-80% of RA patients in hospital series (51); the sensitivity for RF in RA varies from 19% to 53% and the variation in specificity is 91.7-98.6% (276). RF was included in the 1987 revised criteria for the classification of rheumatoid arthritis by the American Rheumatism Association (ARA) (14).

Citrulline is an amino-acid that is incorporated into proteins during inflammation. The presence of ACPA, as RF, portends a more aggressive disease course along with a higher degree of systemic inflammation, and can be detected years before RA onset (26, 277). Its specificity is higher than RF, and reported ranges of diagnostic sensitivities and specificities are 39-94% and 81-100%, respectively (19). In 2007, ACPA antibodies were included in the European League Against Rheumatism (EULAR) guidelines for the diagnosis of early RA, and in the American College Rheumatology (ACR) criteria for RA classification (61, 209).

There are data suggesting an association between RF and/or ACPA and risk for CVD and mortality in RA and also in healthy individuals. This association can partially be explained by cigarette smoking as smoking is associated with RF and ACPA production. However the heightened CVD and mortality risk does not appear to be linked only to smoking neither in the general population nor in patients with RA (145, 161, 360, 362).

In the general population, RF is an independent risk factor for ischemic heart disease, all-cause and/or CVD mortality, at least in men (3, 94, 348). In a population-based longitudinal study, participants with “false-positive RF” titers of ≥ 128 have been found to have a 74% increased risk of CVD deaths (161). The presence of RF, but not ACPA antibodies, has recently been confirmed to be a significant predictor of CVD events and mortality in both those with and those without rheumatic diseases, which supports the role of immune dysregulation in the etiology of CVD disease (207).

In a primary care-based inception cohort of patients with inflammatory polyarthritis of short-disease duration, RF-positive subjects had increased rate of death from all causes as well as death attributed to CVD (150). Such an excess mortality has been confined in recent-onset arthritis in elderly (138). Additionally, in several other studies of patients with RA, RF-positivity has been found to predict all-cause and CVD mortality (50, 146, 148, 274, 390). Then, in women with RA, presence of a positive RF has been reported to be associated with increased mortality and relative risks of incident CVD events (235, 326). Here also, RF- and/or ACPA-positivity, among other markers of RA severity, together with traditional CVD risk factors are likely to contribute to prediction of CVD events in RA, and increasing frequency of both types of factors are associated with greater risk (327).

ACPA antibodies has been associated with the development of ischemic heart disease, odds ratio (OR) 2.8 (95% CI 1.19-6.56), and also, with higher mortality rates, OR 1.72 (95% CI 1.01-2.91) (219). The excess risk of premature death seems to be most marked for patients with inflammatory polyarthritis and RA in conjunction with ACPA, smoking history, and the HLA-DRB1*01/*04 gene (108).

Strikingly few studies have examined the relationship between serological markers and findings of subclinical atherosclerosis, but the results of one study does imply that patients with RA who are positive for ACPA have enhanced subclinical atherosclerosis compared to those who are not (135).

However, there is discordance in published data addressing relationship of autoantibody status and atherosclerotic outcomes, with negative results reported in several studies (34, 80, 104, 139, 169, 260, 356).

1.5.3 Measures of RA disease

There are six core outcomes which are recommended in clinical studies of RA, i.e. disability, pain, the patients global assessment of general health, the physician global assessment of disease activity, swollen joint count and tender joint count (1). Because of heterogeneity of the disease, a set of variables is preferred to one single variable to assess disease activity (372). Currently, no gold standard measure exists for RA disease activity.

1.5.3.1 DAS28, a composite measure of disease activity

In daily practice and clinical trials, disease activity in RA is usually measured by the composite index Disease Activity Score 28 (DAS28), consisting of a 28 swollen joint count (range 0-28), a 28 tender joint count (range 0-28), ESR, and the patients general health assessment on a visual analogue scale (range 0-100) (272). The DAS28 has a continuous scale ranging from 0 to 9.4, and the DAS28 < 2.6 classifies as disease remission, the DAS28 $\leq$ 3.2 as low disease activity, the DAS28 between > 3.2 and $\leq$ 5.1 as moderate activity, and the DAS28 >5.1 as high activity (121). This score has been shown to correlate with other indices of inflammation and disability (272).

When exploring utility of the DAS28, it is important to notice, that the DAS28 was developed and validated to evaluate disease activity status in groups of patients with RA participating in clinical trials to reflect a clinical meaningful target of anti-rheumatic treatment (low disease activity), but has not been validated for use in the individual patient. Thus, the reliability of the DAS28 for assessing disease activity in individual patients has been questioned, as overestimation can arise from elevated ESR (due to reasons other than disease activity), or fibromyalgia (352). Another shortcoming of this index is a reduced sensitivity to assess low disease activity or remission (more false-negative cases) (201). Taking into consideration the presence of less objective components in the DAS28 and discordance between the DAS score and the physician's assessment of RA activity (389), evaluation of not only the DAS28 but also its individual components along with a full physical evaluation could be recommended.

Evidence on the value of the DAS28 in prediction of future CVD events and mortality outcomes in RA is limited, but some data are available. Thus, in male veterans with RA, DAS28 $\geq$ 5.1 predicted subsequent major adverse CVD events, HR 1.3 (95% CI 1.1-1.6), independent of traditional CVD risk factors (22). Several studies have also found the association between the DAS28 (as a single measure at inclusion or a

cumulative measure) and risk of CVD and/or mortality in RA (169, 274), while other studies have reported a lack of association (104, 275).

1.5.3.2 HAQ, measure of physical function

Functional assessment is commonly made using the self-reported Stanford Health Assessment Questionnaire (HAQ). The Swedish version scores the ability to perform 20 daily activities grouped into 8 categories, and ranges from 0 (least disability) to 3 (most severe disability) (97). Correlation for reliability, i.e. the ability to reproduce results, ranges from 0.87 to 0.99, and for validity, i.e. the degree to which it measures what it is intended to measure, ranges from 0.71 to 0.95 (37). The HAQ score correlates moderately with disease duration, joint pain, other disease activity measures, educational status and low socioeconomic background, as well as female sex and muscle strength (156, 370, 388).

HAQ is a useful instrument for groups of patients even though individual patients show great variation over time, and upward reappraisal of functional ability with increasing time (388). As with any instrument, the HAQ score has limitations, such as the lack of sensitivity to small changes at the ends of the spectrum and the floor and "ceiling effects" (in certain circumstances, the score cannot worsen). Also, absolute changes in the upper range and in the lower range are probably not comparable, i.e. the score data are not truly continuous (37). The HAQ score is affected by both reversible and irreversible components of the disease process, and longstanding disease lessens the potential for improvements in score because of irreversible damage. Thus, patients with RA may derive benefits from treatment that are not reflected in the HAQ score because of irreversible joint damage (37).

The HAQ score has proved to be a strong predictor of all-cause and/or CVD mortality (34, 40, 150, 234, 266, 390, 398), probably to the same extent in both RA and the general populations (322). Moreover, it has been suggested that the 1-year HAQ score may perform as a stronger independent predictor of mortality than the baseline HAQ in RA (109, 398). On the contrary, data on the role of the HAQ score for adverse CVD events are limited, with few reported findings of positive association for the HAQ scores $\geq$ 1.38, or $\geq$ 2 (171, 327).

The inconsistency in reports of mortality and CVD outcomes in relation to the HAQ score in RA invokes the possibility that the functional score may provide indirect measure of the patients' overall health status/age/co-morbidities, at least in established RA disease, and provide less information on cumulative inflammation.

1.5.3.3 Measure of pain

Pain can be measured by a visual analogue scale (VAS), range from 0 (none) to 100 mm (extremely severe). A number of studies have established that data from self-reporting visual analog scales are reproducible, and the score of 10-25 mm VAS may indicate normal status (321).

A visual analog pain scale was initially used in psychology by Sigmund Freud and others since the early 1900s; in rheumatology, the use of a pain VAS was developed in the in the late 1970s (321). Pain follows the same pattern of development as other variables of disease activity in groups of patients with RA, after initial improvement, pain scores slowly deteriorate over the years. In early severe RA pain reflects the nociceptive effects of local inflammation and is significantly correlated with patients'

global health assessment, HAQ and laboratory variables (ESR, CRP) (194, 321). Although significant correlations can be seen between pain scores and the radiographic scores of small joints, the strongest associations are seen between pain score and the scores for functional status and the psychological constructs of anxiety, depression, helplessness, and lack of self-efficacy (105, 296, 321, 323). A quantitative assessment of pain is generally recommended to be carried out at each visit in routine rheumatology care, along with an assessment of functional disability, the global status, and other patient variables.

Although controlling pain is one of the targets of successful treatment (379), unfortunately, many RA patients continue to suffer pain despite a range of treatments with DMARDs and symptomatic drugs (6). In the general population, pain is suggested to be one of the modifiable risk predictors for premature mortality (10, 324). If pain and changes in the pain scores in the course of RA exert an effect on future adverse CVD outcomes and can be used for prognosis deserve investigation.

1.5.3.4 Key considerations

Prospective cohort studies including several inflammatory and RA disease related markers are necessary to assess the value of more complex models as predictors of outcomes in RA. Models involving several biomarkers and clinical markers may result in a biomarker signature capable of predicting and monitoring outcomes important to patients. In turn, this has the potential to allow tailoring of treatment regimens and prevention to groups of patients according to their biomarker signature and would truly represent personalized medicine.

1.6 ANTI-RHEUMATIC THERAPIES AND CARDIOVASCULAR RISK

There is a complicated relationship between cardiovascular conditions and inflammatory activity, disease severity, and drug therapies used in RA, which is not fully understood (21, 190). In recent years considerable attention has been drawn to the ability of anti-rheumatic drugs to modulate CVD and mortality risk in RA via specific targeting of the inflammatory process implicated in atherogenesis and atherothrombosis. Although results from observational studies vary (128, 197), and the current evidence is not definitive, there is a real possibility that better disease control could confer survival benefits (54, 197, 236).

Controlling systemic inflammation may reduce risk of CVD and premature mortality, but therapies themselves may contribute to atherogenesis and/or mortality due to unfavorable effects (241). Despite frequent use of NSAIDs and also glucocorticoids (GCs) by patients with RA, and concerns surrounding CVD risk in association with their use in the general population (162, 330, 354, 376), this issue has not been extensively studied in patients with RA. Though some observational studies could not confirm NSAIDs- or corticosteroids-associated CVD risk in RA (30, 147), hitherto, the published data do not provide firm conclusions about benefit/harm profile of these drugs in a condition of a marked inflammatory burden.

Use of any DMARDs (169, 243, 342, 362, 368), in particular methotrexate (MTX), and/or biologic agents (4, 23, 54, 153, 171, 233, 380, 381), may halt CVD and reduce CVD mortality; this is again thought to be due to effective long-term control of systemic inflammation. Thus, in severe RA, non-responders to MTX treatment have been documented to have a poor survival prognosis with >4-fold increased mortality compared with the general population, while patients who respond to MTX had only a moderately increased mortality rate (197). Also, a risk reduction in the

incidence of AMI has been found in responders compared to non-responders to TNF-α blockade (87).

A recent meta-analysis has explored the relation of MTX therapy with CVD in patients with RA, psoriasis, or polyarthritis, and in observational studies the use of MTX was associated with a 21% lower risk for total CVD (95% CI, 0.73-0.87) and an 18% lower risk for AMI (95% CI, 0.71-0.96). Importantly, stronger associations were observed in studies that adjusted for underlying disease severity (233). According to a systematic literature review, the effect of anti-TNF-α agents on reduction of CVD in RA has not been as consistent as in studies of MTX (380). Still, the meta-analysis of cohort studies, but not randomized controlled trials (RCTs), has found that anti-TNF-α therapy was associated with a reduced risk for all CVD events, pooled adjusted RR 0.46 (95% CI, 0.28-0.77) (23).

Although National Registries provide comforting data about CVD safety of anti-TNF-α therapies (87, 217), they cannot adequately assess the actual risk, as these drugs are administered to patients with no cardiac dysfunction. An increased risk of heart failure with TNF-α blockade has been documented (200, 304).The common shortcomings of many studies which have investigated the impact of drugs on risk of CVD and mortality in RA are highly different patient populations, various inclusion criteria and definition of the outcomes, inadequate control for confounding factors and response to treatment, various total duration of therapy and cumulative drug exposure in conjunction in different stages of RA disease, and confounding by indication. However, despite limitations of the studies, the results yield some important, conclusive information which raises the possibility of specific interventions to reduce CVD morbidity and premature mortality in RA. The hypothesis to be tested is that tight control of disease activity would dissociate the inflammatory clinical variables at baseline from their predictive value, i.e. “dissociation of risk and reality” (316). The EULAR recommendations highlight the need for rheumatologists to treat RA patients with active disease early and effectively, identify patients at-risk for coexistent risk factors and treat them actively or with preventative measures accordingly, and this applies especially to cardiovascular co-morbidity (258).

2 AIMS

i. To explore whether treatment with biologic agents in patients with RA, as TNF-α blockade and anti-CD20 therapy influences CVD biomarkers, such as apolipoproteins, oxidized LDL, and the atheroprotective IgM antibodies against phosporylcholine (anti-PC IgM)

ii. To examine the associations of established CVD risk factors, RA disease related factors and novel cardiovascular biomarkers, such as apolipoproteins, oxLDL and anti-PC IgM with measures of carotid atherosclerosis

iii. To analyse whether the aforementioned novel cardiovascular biomarkers, RA disease factors and measures of carotid atherosclerosis are linked to incident CVD events during prolonged follow-up

iv. To estimate the associations of disease related factors and anti-PC IgM antibodies the first years after diagnosis of RA with incident CVD morbidity and all-cause mortality, and to determine whether the impact of these factors differ in patient groups depending of age at onset of RA

3 PATIENTS AND METHODS

3.1 PATIENTS

The studies were of prospective observational design. At inclusion, all participants had a RA diagnosis according to the ACR criteria (14). Concomitant disease-modifying anti-rheumatic drugs (DMARDs) were chosen by the treating physicians in accordance with the current recommended treatment strategy in Sweden, at that time, the strategy implied initial monotherapy with DMARDs and early use of low-dose oral glucocorticoids, with "step-up" combination therapy reserved for more severe disease.

All study participants provided written informed consent. The studies were approved by the Regional Ethic committee in Stockholm, Sweden, and were performed in accordance with the Declaration of Helsinki.

Paper I. A total of 215 outpatients with established RA disease were identified from a prospective cohort of patients registered in the local database, including all patients treated with biologic agents at the Rheumatology Department, Karolinska University Hospital Huddinge. They had started their treatment with TNF-α blockade, or rituximab between January 2000 and October 2007, and were enrolled consecutively into the study in case they had remained on the treatment for at least one year, and if they had been biologic-naïve earlier (in case of anti-TNF-α treatment). At that time, rituximab was used mainly in patients that had failed on anti-TNF-α therapy, and 68% of these patients had been taken anti-TNF-α treatment earlier. One course of rituximab was given during the study period.

Of the patients, 162 had been treated with anti-TNF-α (etanercept, n=60; infliximab, n=60; adalimumab, n=42), and 53 with rituximab. The patients were aged 57.9 (12.4) years, 75% of them were females, 85.6% RF-positive, and 87% had erosive disease after a mean disease duration of 8.5 (5-15) years. At the time of initiation of biological therapy, they had a median CRP of 24 (10-46) mg/l, a mean ESR of 43 (24) mm/h, and a mean DAS28 of 5.7 (1.1). Of the patients, 70% used concomitant MTX, and 45% oral glucocorticoids in dosage of 7.5 (5-10) mg daily.

*Papers II-IV***.** We used the study population from the early RA cohort, BARFOT (Better Anti-Rheumatic FarmacO-Therapy). The BARFOT is a six-centre prospective observational study covering both urban and rural patient's referral areas. The BARFOT was designed and started in the early 90th in order to investigate clinical and therapeutic aspects of early RA in the long term (344). The inclusion criteria for the BARFOT were RA diagnosis according to the ACR definition (14), age >18 years and disease duration ≤ 12 months. After baseline evaluation, regular follow-up assessments according to the study protocol had been conducted. The BARFOT database is regularly updated.

Papers II-III. In these studies, the study population was 114 patients from the Rheumatology Department Huddinge (one of the BARFOT's centre), who between June 2000 and March 2004 had been followed for five years since RA diagnosis and were aged < 70 years at BARFOT inclusion. Of 203 eligible patients, 114 individuals were available and willing to undergo high-resolution B-mode ultra-sonography of the carotids. Thus, 89 subjects were not included because of unwillingness or because they had moved. These non-participants did not differ from the patients included in the study in age, sex, baseline DAS28 and functional disability. The follow-up lasted

5 years (*paper II*) and 12 (2.9) years (*paper III*). The main characteristics of the patients at the study entry are shown in the table below.

Paper IV. The study base consisted of all BARFOT patients with early RA who were consecutively recruited from 1993 through 1999, n=861. Individuals with prevalent CVD at RA diagnosis, n=113, and patients who were < 20 years of age at inclusion, n=7, were excluded. The final study population consisted of the 741 patients. The median observation time was 13 (12-14) years. The patient's characteristics at the study entry are summarized in the table.

Table. Patient characteristics at time for inclusion in the studies

	Papers II and III 114 patients	*Paper IV* 741 patients
Age, years	50.6 (11.2)	55.0 (14.7)
Females, %	68	68
Smokers, ever, %	68	59
Diabetes mellitus, %	2	5
Hyperlipidemia, %	2	1
RF-positivity, %	59	60
Disease duration, months	6 (3)	6 (3)
CRP, mg/l	24 (11-46)	19 (6-46)
ESR, mm/h	43 (26)	36 (26)
DAS28	5.4 (1.2)	5.1 (1.3)
HAQ score	1.2 (0.6)	1.0 (0.6)
Therapies:		
methotrexate, %	19	44
glucocorticoids, %	35	51

3.2 METHODS

3.2.1 Disease assessments

Clinical and routine laboratory examinations were conducted at treatment initiation with biologic agents and after 3, 6 and 12 months (*paper I*), at inclusion to the BARFOT study and after 3, 6, 12, 24 and 60 months (*papers II-III*), and at inclusion to the BARFOT study and after 12 and 24 months (*paper IV*). Demographics, medical history, smoking status, anthropometric data and disease measures were obtained from the medical records (*paper I*) and the BARFOT database (*papers II-IV*).

RA disease activity was calculated using the Disease Activity Score for 28 joints (DAS28) with ESR (272). A DAS28 < 2.6 was classified as disease remission (120) (*paper I*). Functional status was self-assessed by the validated Swedish version of the Stanford Health Assessment Questionnaire (HAQ), range from 0 to 3 (97). Pain was measured by a visual analogue scale (VAS pain), range from 0 to 100 mm.

Information on medication, cardiovascular risk factors, the presence of co-morbid conditions were collected through the BARFOT registry and medical records, and also, through discharge diagnoses from hospital admissions, ICD-9 and ICD-10 codes (*paper IV*). At each follow-up visit information on medication was up-dated, and the regular use of DMARDs, biologic agents and glucocorticoids was considered if it was reported at least during 6 months throughout the follow-up (*papers II-IV*).

Papers II-III. At BARFOT inclusion, a patient was defined as having a pre-existing CVD if angina pectoris, acute myocardial infarction (AMI), coronary surgery or ischemic stroke were diagnosed by a physician according to current criteria and documented in patients' medical history.

Paper IV. Prevalent CVDs at RA diagnosis were tracked through medical records and administrative data sources, and included AMI, angina pectoris, heart failure, atrial fibrillation, peripheral arterial disease, ischemic stroke and transient ischemic attack.

3.2.2 Assessment of traditional cardiovascular risk factors

Smoking status was self-reported as daily ever smoking (current or past) or never smoking. Hypertension was considered if prescription of anti-hypertensive medication and/or blood pressure above 140/90 mm Hg (*paper I-III*) or documented diagnosis (*paper IV*). Diabetes mellitus was defined as history of diabetes and/or prescription of anti-diabetic medication (*papers I-IV*). Dyslipidaemia was defined as: prescription of lipid-lowering drugs (*paper I*) and/or serum apoA1 <1.25g/l for women and <1.15g/l for men, apoB ≥ 0.9g/l for both genders, apoB/apoA1 ratio ≥ 0.6 for women and ≥ 0.7 for men (363) (*paper II*); total cholesterol (TC) ≥ 5.0 mmol/L, or low-density lipoprotein (LDL) ≥ 3.0 mmol/L (*paper III*); documented diagnosis (*paper IV*).

3.2.3 Laboratory assays

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were analysed with routine methods. CRP was measured by a non-high sensitive assay and low levels were reported as < 10 mg/l. Rheumatoid factor (RF) was measured by agglutination test where a positive titre was > 1/20 (*papers I-III*).

Venous non-fasting blood samples were also aliquoted immediately and collected for storage (–70°C) in a bio-bank for the following analyses. Sera at study enrolment were analyzed for RF IgM using the Serodia agglutination test, (Fujirebio, Tokyo, Japan), a titre of > 20 IU/ml was regarded as positive, and anti-citrullinated peptide antibody (ACPA), using the ELISA CCP2 test, (Euro-Diagnostica, Malmö, Sweden), positive ACPA was defined as a titre > 25 U/ml (*paper IV*).

Apolipoprotein A1 (apoA1) and B (apoB) were determined with Modular Analytics P, Roche Diagnostics (*paper I*), and with Synchrone LX from Beckman AB (*papers II-III*) by immunoturbidimetry at the Study centre for laboratory medicine, Karolinska University Hospital, Stockholm, Sweden. The apoA1 reference intervals, given by the Study centre, are 1.10-2.10 g/l for women and 1.10-1.80 g/l for men. The apoB reference interval is 0.50-1.50 g/l for individuals younger than 40 years and 0.50-1.70 g/l for those who are of 40 years and older.

OxLDL (*papers I-III*) and anti-PC IgM determinations were carried out using a commercial enzyme linked immunoassay (ELISA) kit (Mercodia AB, Uppsala, Sweden), and Athera CVDefine^{TM} ELISA kit (Athera Biotechnologies AB, Stockholm, Sweden) according to the protocols provided by the manufacturers and essentially as described earlier (100, 314). Coefficients of variations were < 6.2% for oxLDL, and < 7% for anti-PC IgM.

3.2.4 Carotid intima-media measurements

Papers II-III. High-resolution B-mode ultrasonography of the carotids was performed approximately 5 years after RA disease onset, between June 2000 and March 2004. The right and left carotid arteries were examined with a duplex scanner (Aspen, Acuson, Mountain View, Ca, USA) using a 7 MHz linear array transducer. The far wall of the common carotid artery (CCA), 0.5 to 1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of the carotid intima-media thickness (cIMT). The cIMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo and was measured in the region free of atherosclerotic plaques. For right and left CCA the mean values of the cIMT within the 10 mm long section were estimated. Then, the mean cIMT [(right + left)/2] was calculated. Carotid plaque, defined as a localized intima-media thickening of greater than 1 mm and at least a 100% increase in thickness compared with adjacent wall segments, was screened for in the common, internal and external carotids (203). Plaque occurrence was classified as the absence of plaque (none), the presence of unilateral plaque and the presence of bilateral plaques. The carotid measurements were made by two certified ultrasonographers. The intra-reader coefficient of variation for cIMT was 3.2%.

3.2.5 Assessment of CVD outcomes

Paper III. The study outcome was the first ever CVD event occurring during the follow-up. Information on incident CVD event was obtained retrospectively and validated through a structured review of the medical records. The CVD events were predefined as any incident acute myocardial infarction (AMI, a diagnosis based on history, electrocardiogram [ECG], and/or echocardiography together with typical enzymatic pattern and/or angiography), angina pectoris (registered as history of typical chest pain with compatible ECG or myocardial scintigraphy, stress ECG or stress echocardiography and/or angiography), congestive heart failure (CHF) (a recorded diagnosis based on history of at least one episode of symptomatic heart failure with continued dyspnoea, New York Heart Association class 2-4 together with typical radiography and/or echocardiography) or ischemic cerebrovascular event (both ischemic stroke and transient ischemic attack diagnosed with a typical clinical picture with neurological deficits and/or verified with computerized tomography and/or magnetic resonance imaging).

Paper IV. The outcomes were a composite incident CVD event (i.e. fatal or non-fatal myocardial infarction, cardiac arrest, angina pectoris, coronary bypass grafting or percutaneous coronary intervention, peripheral arterial disease, angioplasty or other vascular surgery, fatal or non-fatal stroke and transient ischemic attack) and all-cause mortality. The observation period started between July 1993 and October 1999, i.e. when the patients were included in the BARFOT-study. The follow-up lasted until occurrence of the first-ever incident CVD event, death, or to December, 2010, whichever came first. Survival confirmation and date of death were obtained from the Swedish National Population Registry. Morbidity data was obtained from the Swedish Hospital Discharge Registry, between January 1987 and December 2010, cause of death from the National Cause of Death Registry, last updated in December, 2010. Registry records were checked for quality of data by comparing with complete medical records.

3.2.6 Statistical analysis

Descriptive analyses of all variables utilized in the data analyses were conducted. Continuous variables were summarized as mean (SD) if normally distributed, or median (IQR) if not. Categorical variables were presented as frequencies (percentages). oxLDL and anti-PC IgM levels were dichotomized by percentiles, or determined as continuous variables as indicated.

Clinical features were compared, using the t-test, Mann-Whitney U-test or Wilcoxon rank test for continuous variables, Kruskal-Wallis test for multiple samples, and the chi-square or the Fisher exact tests for categorical variables. Spearman test was used to examine correlations. Log transformations were undertaken if required to fulfill assumption of normality.

Area under the curve (AUC) using the trapezoidal rule was calculated for the core disease measures assessed at inclusion and after 1 and 2 years, and reduction (Δ) between inclusion and 1 year after enrolment (*paper IV*).

A 2-tailed value of $p < 0.05$ was considered significant. All statistical analyses were performed using STATISTICA (Stat Soft Scandinavia AB, Tulsa, OK, USA) and IBM SPSS (SPSS Inc., Chicago, IL).

Paper I. McNemar's test was employed for dichotomous outcomes in analysis of differences in one sample at different time-points. To analyse longitudinal changes in IgM anti-PC we used repeated measures ANOVA in the complete dataset with probabilities for post-hoc test for between and within group comparisons at different time points. A multivariate approach was applied as indicated in order to elucidate the possible effect of intergroup differences.

The fact of intermittent missing data of outcome variables, like anti-PC, oxLDL and apolipoproteins after 6 or 12 months observations (overall, 13% and 7.8% missing data, respectively), was a concern. Because of high variation of the primary objectives and skewed distribution of the variables, we chose not to use imputation techniques while taking into consideration reduced power of our analyses. No statistically significant differences were observed between patients with complete and missing data in regard to age, sex, history of smoking and co-morbidities, DMARDs and glucocorticoid treatment, usage of lipid-lowering medication and baseline features of RA disease activity.

Paper II. To investigate the association between study outcomes and different risk factors, we applied a series of univariate regression analyses. Then, we constructed multivariate regression models (linear for continuous outcome and ordinal logistic regression for categorical outcome) to detect a combination of significant traditional factors, and then, to this base model we added studied factors one by one, entry procedure with $p < 0.10$ criterion.

Paper II-III. To evaluate the relationship between variables measured repeatedly over first five years after RA diagnosis and study outcomes, we applied mixed linear modeling for continuous variables and GEE (generalized estimating equations) for dichotomized variables with two between-group factors (plaque presence or not, occurrence of CVD event or not) and one within-group factor Time (0, 3, 12, 24, 60 months). Time was modeled as a categorical variable because of unequally spaced time intervals. To allow for the means of the response variables to differ between CVD and none CVD groups as time progresses, an interaction term with factor Time was included in the models. The best fit model was chosen according to the covariance structure and the smallest value of the Likelihood Information Criteria.

For these longitudinal analyses, in *paper III*, 9 cases with CVD ahead of RA onset were excluded.

Paper III-IV. The outcomes' incidence rates (with the 95% confidence interval (CI) for a Poisson count) were presented as events per 100 person-years at risk, performed separately for the whole follow-up period and for the observation period after carotid ultrasonography examination (*paper III*), and for the study outcomes apart (*paper IV*).

The Kaplan-Meier method was used to describe the outcome-free survival for the groups stratified by cIMT in tertiles or carotid plaque occurrence (*paper III*), and to assess the relationship between anti-PC IgM levels, dichotomized by tertiles, and the outcomes (*paper IV*). Log-rank (Mantel-Cox) statistic was applied to compare the survival functions obtained from the groups.

The main analyses tested Cox proportional hazard models, with predictors of interest as the independent variables. Unadjusted models were tested first, followed by multivariate models, entry procedure with $p < 0.10$ criterion, which were adjusted for age (*paper III*), and also for gender, smoking at enrolment, presence of hypertension, diabetes or hyperlipidemia (*paper IV*) as fixed-in-time binary variables measured up to the date of the outcome or the end of the follow-up (*papers III-IV*). Stratified analyses within age groups (<65 years old, ≥ 65 years old at RA onset) were also done (*paper IV*). Cases with a CVD event within five (*paper III*) or two *(paper IV*) years after inclusion were excluded from analyses if required for the correct interpretation. A 95% CI for hazard ratio (HR) was used to estimate the likely range of effect for the population coefficients.

4 RESULTS AND DISCUSSION

4.1 APOLIPOPROTEINS AND OXLDL

4.1.1 *Influence of biologic agents*

Paper I. In established RA disease, levels of apolipoproteins did not differ statistically between the groups of patients ahead of treatment with anti-TNF-α or rituximab. The mean apoA1 was normal, >1.29g/l, but the mean apoB was elevated, >0.88 g/l, resulting in a high mean of pro-atherogenic apoB/apo1 ratio, >0.69, in all study groups. Overall, levels of apoA1 increased throughout the treatment period, and towards 12 months apoA1 had stabilized at higher levels than at baseline. At the end of follow-up, i.e. one year after commencement of biological therapy, apoA1 had increased, on average, by 8.5% on etanercept, by 2.3% in the total anti-TNF-α group, and by 6.7% on rituximab, with no significant inter-group difference. The apoB and the apoB/apoA1 ratio remained relatively stable throughout the whole study period.

At baseline, oxLDL levels did not differ between the treatment groups. After 3 months of therapy, oxLDL had increased significantly both on anti-TNF-α and rituximab treatments, on average, by 4% and 6% respectively, but this increase waned thereafter.

Associations between changes in lipids and inflammatory markers during the period of follow-up were similar in patients on TNF-α blockade and those on rituximab. In the whole study population, a low to moderate negative association was observed between absolute change (Δ) in apoA1 levels and ΔCRP at 6 months, r= -0.23, p=0.002, and at 12 months, r= -0.27, p=0.0001. Similarly, ΔoxLDL levels were inversely correlated with ΔCRP: at 3 months, r= -0.18, p=0.009; at 6 months, r= -0.26, p=0.001, and at 12 months, r= -0.24, p=0.001. We did not detect any significant associations between the apoB, the apoB/apoA1 ratio and changes in disease activity during follow-up.

The changes in the levels of apolipoproteins and oxLDL were documented during a period of an overall decrease in RA disease activity and similar reductions in DAS28 in patients treated with anti-TNF-α and rituximab.

Conclusions and discussion: Both anti-TNF-α and rituximab treatments improved the apoA1 levels but transiently worsened the oxLDL status. Likely, these findings correspond to the increased anti-atherogenic lipids but also the trend of increased pro-atherogenic lipids, such as total cholesterol and triglycerides, in relation to improved inflammatory measures reported in other studies (59). Importantly, the effect on lipids was not agent specific but rather a result of a reduced disease activity. Consistent with earlier reports the magnitude of changes in apoA1 was fairly mild (268).

The pattern of lipid levels during inflammation is pro-atherogenic and believed to contribute to atherosclerosis, especially in chronic inflammatory diseases such as RA. TNF-α inhibitors and CD20 targeted therapy can reduce disease activity and structural damage, and thus, could also diminish the increased CVD risk associated with RA by attenuating systemic inflammation linked with atherogenesis (87, 172).

Published reports on apolipoprotein levels after short- and long-term treatment with anti-TNF-α in patients with RA are contradictory, and with regard to lipid changes, rituximab has been poorly studied (69, 268). It is uncertain, whether biologic agents influence lipid homeostasis by direct mechanisms or via dampening of inflammation. As to TNF-α, accumulated data indicate that it could directly perturb the lipid

metabolism through several mechanisms, such as suppression of free fatty acid uptake and promotion of lipogenesis, induction of lipolysis, inhibition of lipid-metabolism-related enzymes activity, regulation of cholesterol metabolism and regulation of adipocyte-derived adipokines (52).

The reported increase in the apoA1 levels is limited, therefore, it is uncertain whether quantitative raising apoA1 levels *per se*, independent of other changes in lipid and/or non-lipid risk factors, can have clinical significance for reduction the risk of CVD. Risk modification may be due to the correction of inflammation and similar effects might be found with other treatments (69). Given the finding of similar efficacy of TNF-α inhibitors and rituximab, and the correlation between changes in lipids and inflammatory markers in our study, it seems likely that major effect could be related to the suppression of inflammation, which is associated, in turn, with increments in apoA1 and long-term unaltered levels of oxidized LDL.

The clinical impact of these findings is unclear, and further studies are needed to clarify the role of the lipid changes on cardiovascular morbidity in RA. Also, studies addressing qualitative lipid disorders during anti-rheumatic therapy are warranted.

*4.1.2 **Associations with carotid atherosclerosis and CVD***

Paper II. In the early RA patients, the levels of apolipoproteins had not changed at the 5-year assessment compared with baseline, whereas the oxLDL levels had increased, p=0.000.

Controlled for age, gender, smoking status and history of CVD/ hypertension/diabetes, longitudinal levels of log apoA1 were inversely associated with cIMT (ß= -0.24, 95% CI -0.47; -0.02, p=0.047), whereas log apoB/apoA1-ratio showed a positive association (ß=0.45; 95% CI 0.04-0.85, p=0.030), thus, the reduction in apoA1 by 2.4% (95% CI 0.1-4.6%) or the increase in the apoB/apoA1 ratio by 4.6% (95% CI 0.3 – 8.9%) corresponded to the increase in cIMT by 0.1 mm. Neither levels of log apoB nor oxLDL over-time were significantly related to cIMT.

Higher levels of apoB and the apoB/apoA1-ratio during the follow-up proved independent associations with detection of carotid plaque after five years of RA disease, p=0.002, p=0.026, respectively. Then, higher levels of oxLDL over-time tended towards a positive association with the plaque presence, p=0.079.

Paper III. The measures of apolipoproteins at baseline and over the first five years of RA disease failed to demonstrate significant associations with the CVD outcomes during the follow-up of a mean 12 (2.9) years. By contrast, levels of log oxLDL over-time, but not at baseline, were higher in individuals who experienced a subsequent CVD event compared with those who did not, p=0.027.

Conclusions and discussion: These results suggest that apolipoproteins are independent predictors of cIMT and carotid plaque in patients with RA. To date, published data are insufficient to conclude if changes in apolipoproteins, especially in apoA1, mirror changes in traditional lipids, and if apolipoproteins are more susceptible to fluctuation due to inflammatory changes, or if apolipoproteins may be more useful for absolute CVD risk estimation in patients with RA (351).

A systematic review and meta-analysis have shown that for an absolute cIMT difference of 0.1 mm the future risk of myocardial infarction increases by 10% to 15%, and the stroke risk increases by 13% to 18% in the general population (220). In this regard, our results suggest that even small changes in apoA1 and the

apoB/apoA1-ratio, by mean of 2.4% and 4.6% respectively, might correspond to a clinically significant risk of future CVD.

Importantly, despite effective control of inflammation, we documented here an increase in levels of oxLDL during five years of follow-up after RA disease onset. This increase of oxLDL might influence both progression of atherosclerosis and the RA disease *per se*. However, our results were not conclusive about association between oxLDL levels and carotid ultrasound measurements.

Traditional risk factors may behave differently in RA and in the general population. Thus, in the large AMORIS-study the predictive value of lipids for AMI and ischemic stroke were not consistent in the RA patients (302). However, the non-associations of apolipoproteins and the CVD outcome in our study do not exclude a potential influence of traditional risk factors for underlying pro-atherogenic mechanisms. Thus, the patients who experienced CVD events after RA-onset, compared with those who did not, were more frequently classified with hyperlipidaemia or gained increasing levels of oxLDL between the study entry and the 5-year assessment.

In the general population, increasing plasma oxLDL levels are associated with increased risk for CVD (343) and predictive for CVD, independently of traditional lipids and traditional cardiovascular risk factors (230), as well as for rupture-prone atherosclerotic plaques (206). Additionally, serum oxLDL are raised in RA and correlate with disease activity independently of other inflammatory markers, suggesting the importance of oxLDL in a chronic inflammatory condition *per se* (187, 393). Taken together, oxLDL may have significance for CVD outcome probably due to plaque instability, but further studies are needed.

4.2 ANTI-PHOSPORYLCHOLINE ANTIBODIES

4.2.1 Influence of biologic agents

Paper I. At initiation of biological therapy, there was no statistically significant difference in the anti-PC IgM levels between patients started on anti-TNF-α and rituximab, or between RF-positive and RF-negative patients. On anti-TNF-α therapy, the levels of anti-PC increased significantly already after 3 months, and overall, by 26% after 12 months, $p<0.001$, while the levels decreased by 14% on rituximab, $p=0.023$. At all time-points of follow up, the anti-PC levels were significantly higher in the anti-TNF treated vs. the rituximab treated patients, $p<0.001$, independent of age, gender and baseline disease characteristics.

At entry, there were no significant correlations between the anti-PC concentrations and levels of inflammatory markers, disease characteristics or apolipoproteins. Nonetheless, on both anti-TNF-α and rituximab, patients achieving remission at the end of the study (DAS28<2.6), compared with those not in remission, had significantly higher baseline anti-PC levels, $p=0.007$ and $p=0.041$, respectively.

Conclusions and discussion: Based on the findings summarized in introduction, it has been proposed that the low levels of anti-PC IgM represent an immune-deficient state of depressed anti-inflammatory capacity associated with an increased risk of chronic inflammatory diseases, for example, atherosclerosis. Also, the low anti-PC IgM levels may promote disease activity and disease outbreaks in auto-inflammatory condition through insufficiency in anti-inflammatory and anti-apoptotic properties (122). In this aspect, the finding that higher anti-PC levels were more common among individuals achieving remission in RA, than among patients not in remission after one year´s use of biologic agents, raises the question whether measure of anti-PC could

identify patients prone to a good therapy response, whether treatment with anti-PC may intensify effect of other medications, and whether anti-PC *per se* may have a positive effect in RA.

The mechanism by which the anti-TNF-α therapy was associated with increasing anti-PC IgM levels in the present patients with RA is not clear. One possibility is that TNF-α has a direct inhibitory effect on B cells which produce anti-PC (unpublished data), but it is also possible that anti-PC is increased indirectly as a consequence of decreased inflammatory burden in general.

While anti-TNF-α treatment has been extensively studied in the context of CVD, little is known about the risk of CVD following rituximab in rheumatic disease (250). In humans, treatment with rituximab induces an almost complete depletion of circulating B cells that usually lasts for 6 to 9 months, and in humans and primates persistent partial B-cell depletion may be found also in bone marrow and lymphoid organs (280). Theoretically, depletion of B cells in autoimmune diseases should be limited to conventional B2 cells while sparing regulatory B10 cells and potentially protective B1 cells, producers of natural antibodies (369), but this has not been proven in humans.

The evidence from clinical studies indicates that anti-CD20 therapy targets non-proliferating short-lived memory B cells and their immediate progeny, responsible for production of an array of activity-related autoantibodies, such as RF, ACPA, anti-dsDNA or anti-neutrophil cytoplasmic antibodies. In contrast, long-lived plasma cells, mainly responsible for protective immunoglobulin titers, but only some of the auto-reactive IgG antibodies such as Sm, Ro, La and RNP which do not correlate with disease activity, are not affected by rituximab (90). Thus, after commencement of rituximab, it has been observed a drop in levels of auto-reactive auto-antibodies, and also, of overall serum levels of IgG and IgM, in approximately 5% and 20% of patients, respectively (41, 42, 60, 62, 102), the latter seems to be mostly pronounced following multiple B-cell depletion cycles based on rituximab (75). Interestingly, treatment of RA with anti-TNF-α agents has been associated also with a reduction in the levels of RF and ACPA antibodies probably through downregulation of the production of several inflammatory cytokines and mediators (15).

Taking into consideration potential changes in serum IgM levels after rituximab initiation, it was not unexpected to find decreasing anti-PC IgM levels in the rituximab group, but the increasing levels in the anti-TNF-α patients, despite similar reductions in disease activity, are challenging. As outlined above, probable explanations for the diverse anti-PC courses are inhibition of anti-PC production by TNF-α, an effect that is hampered by TNF-α blockade, and decrease of B-cells anti-PC synthesis by rituximab. Still, it would be preferable to measure parallel both the total levels of IgM and anti-PC IgM in the tested samples, and to examine if levels of total IgM correlates to levels of anti-PC antibodies.

4.2.2 Association with carotid atherosclerosis

Paper II. In the early RA patients, the levels of anti-PC IgM antibodies decreased at the 5-year assessment compared with baseline, p=0.001. In unadjusted regression analyses, the lowest tertile of anti-PC at baseline (≤ 46.6 U/ml) was trend wise positively associated with the bilateral carotid plaques presence five years after RA onset, while the highest tertile of anti-PC at baseline (≥ 87.0 U/ml) corresponded negatively, p=0.054 and p=0.016, respectively. These associations disappeared with

adjustment for age and gender. However, in longitudinal analyses, the low anti-PC tertile during the first five years of RA disease was associated with an enhanced detection of bilateral carotid plaques, p=0.000, independent of age, gender, smoking and history of CVD/ hypertension/diabetes.

Conclusions and discussion: Here we reported that low IgM anti-PC levels over-time had an unfavorable association with the plaque presence after five years of RA disease. Thus, anti-PC IgM may have an independent role in carotid atherosclerosis in patients with RA. This finding is in line with the previous studies where high levels of IgM-antibodies against PC have been a strong protection marker for carotid atherosclerosis development, measured by cIMT, in hypertensive subjects (339), while the low anti-PC levels have been associated with the carotid plaque occurrence in a cross-sectional study of patients with SLE (9).

Further, in the present study the anti-PC IgM levels decreased during the follow-up despite amelioration of inflammation measured by conventional methods. This finding implies that the low anti-PC levels may potentially characterize an immune-deficient state and distinguish ongoing, otherwise unrecognized, inflammation in RA.

4.2.3 *Association with CVD*

Paper III. In the 105 patients with early RA without history of CVD ahead of RA diagnosis (one case contributed only up to 12 months of follow-up before occurrence of a CVD event), the baseline levels of anti-PC IgM failed to show any association with subsequent CVD events. At the same time, an increasing probability of having low levels of anti-PC throughout the first five years after RA diagnosis was demonstrated in individuals who experienced subsequent CVD event compared to those who did not, p=0.002, independent of age, gender and methotrexate usage.

Paper IV. In this inception RA cohort, the levels of anti-PC IgM also significantly decreased during the first two years of follow-up, p=0.000. The levels of anti-PC both at inclusion and after 2 years of observation were lower in participants who experienced a subsequent incident CVD event, then, the latter were more likely to be in the low anti-PC tertile at RA diagnosis (≤ 42.9 U/ml). The levels of these antibodies did not differ between individuals who deceased or survived throughout the study period. In the Kaplan-Meier analysis, the CVD event-free survival, but not all-cause mortality, was shorter in those having the low anti-PC IgM levels, p=0.024. However, multivariate Cox regression tests failed to prove a statistically significant association between anti-PC levels and the study outcomes.

Conclusions and discussion: These findings extend the evidence of an atheroprotective role of anti-PC IgM antibodies, while low levels might be an independent risk marker for CVD outcomes (118, 154, 314). In paper III the levels of natural autoantibodies over-time had a link with incident CVD events, but the initial levels of these autoantibodies did not. It seems possible that direct or indirect effect of chronic systemic inflammation may be of greater importance for development of atherosclerosis and CVD to manifest, but the firm conclusions cannot be drawn from a study of a small sample size. As to results in paper IV, the explanation for the lack of association of natural antibodies and CVD outcomes after adjustment for age, gender and traditional CVD risk factors may imply the significance of conventional or other e.g., disease related risk factors for CVD in the complex inflammatory milieu of RA.

4.3 ASSOCIATION BETWEEN CAROTID MEASURES AND CVD

Paper III. In the 104 patients with early RA (all cases without CVD ahead of RA diagnosis and ahead of carotid ultrasonography at the 5-year assessment), the rate of incident CVD events did not differ as regards cIMT measures, but the rate was approximately 4-fold increased if bilateral carotid plaques were present compared to those without plaque or with unilateral plaque, 4.44 (1.69-7.2) per 100 person-years. Also, CVD event-free survival times were shorter for subjects with bilateral carotid plaques occurrence compared to those without, corresponding to mean (95% CI), 13.9 (12.8-15.0) versus 15.2 (14.7-15.8) years, respectively, since study inclusion, p=0.012 by log-rank test. Then, the bilateral carotid plaques presence, but not cIMT, was associated with strictly atherosclerosis-related CVD outcomes (AMI, angina pectoris, or ischemic cerebrovascular event), age-adjusted HR 6.31 (1.27-31.4), p=0.025.

Conclusions and discussion: Here, we confirmed the association between detection of the bilateral carotid plaques and a poor CVD-free survival in patients with RA. However, the current study did not demonstrate clinical importance of cIMT in survival analyses. The lack of association could depend on low power, few participants followed for more than 10 years, and the lack of carotid ultrasound evaluation at inclusion. Anyway, the presence of traditional risk factors and carotid atherosclerosis (both plaque presence and cIMT) were pronounced in those with CVD events prior to RA onset, while plaque presence and inflammatory-dependent factors were prominent in those with occurrence of CVD events after RA onset. These findings may be in line with non-increase in mortality and CVD reported during the first 10 years of follow-up in inception RA cohorts (17), probably due to a lead time for adverse cardiovascular effects brought about by cumulative inflammation and accumulated disability.

Given discordances in reports on prevalence of carotid plaque and differences in cIMT (196), the evidence for increased subclinical atherosclerosis in RA is not definitive. More importantly, only few studies have explored measures of subclinical atherosclerosis as predictor of CVD events in RA. Reported findings of predictive value of cIMT may be related to composition of the population studied and unaccounted confounders (104, 142). Further larger studies are needed to legitimize the use of subclinical markers of atherosclerosis as outcomes in RA, and whether these markers can indicate CVD prognosis.

4.4 RA DISEASE FACTORS, ATHEROSCLEROSIS AND CVD

4.4.1 Disease measures and carotid atherosclerosis

Paper II. In this prospective study on 114 patients with early RA, baseline levels of disease related factors as ESR, DAS28, tender or swollen joint count and RF positivity failed to show statistically significant association with cIMT and plaque presence after five years disease. The bilateral carotid plaque presence was positively associated with log CRP over-time, but this relation was not verified after adjustment for age, gender, smoking status and history of CVD/ hypertension/diabetes.

Conclusions and discussion: In the present study, neither initial levels nor longitudinal development of tested inflammatory markers revealed associations with carotid measures, after full adjustment. These negative findings might seem to contradict the hypothesis of a correlation between inflammation and progressive atherosclerosis (212). Still, the disease activity in RA is suggested to influence mostly plaque vulnerability but not cIMT measurements or plaque presence *per se* (333). Our

results, however, indicate that the influence of other factors, e.g., traditional CVD risk factors, is substantial and might conceal the effect of other factors. Also, the lack of positive associations may arise from methodological issues such as plaque definition and ultrasound scanning in different arterial segments, non-high sensitive CRP measurement, the frequent use of concomitant glucocorticoids and a small sample size.

4.4.2 Disease measures, CVD and mortality

Paper III. In this study, during a mean follow-up of 12 (2.9) years, the 105 patients with early RA, without history of CVD events prior to RA-onset, experienced 17 CVD events such as AMI, angina pectoris, congestive heart failure or ischemic cerebrovascular event, corresponding to an incidence rate of 1.35 events per 100 person-years (95% CI 0.71-2.0). Improvement in the DAS28, VAS pain and the HAQ score over the first year, but not their initial levels, were age-independently associated with a better CVD outcome, HRs (95% CI) 0.68 (0.5-0.97), 0.97 (0.95-0.99), 0.35 (0.15-0.82), and respectively. Then, the higher HAQ score at the 5-year assessment was associated with a poorer CVD outcome, HR 2.54 (95% CI, 1.31-4.92), independent of age. Other potential confounder factors were not considered as only age, but not gender, ever smoking, hypertension, diabetes mellitus or body weight, was associated with the risk of the outcome in this study.

In longitudinal analyses, log CRP, the HAQ score and VAS pain over the five years of RA disease showed a different pattern of development as time progressed in groups stratified by incident CVD events occurrence or not. Thus, log CRP, the HAQ score, and VAS pain were higher in individuals who experienced a subsequent CVD event, p=0.038, p=0.007, and p=0.030, respectively. This finding was confirmed after further adjustment for age, with the exception of over-time log CRP for which age-corrected association statistically weakened, p=0.069.

ESR at entry or during the first five years after RA diagnosis, and RF presence failed to demonstrate significant associations with the CVD outcome.

Paper IV. In this inception RA cohort incorporating the 741 patients with RA followed >10 years, there were 177 incident CVD events (AMI, cardiac arrest, angina pectoris, coronary bypass grafting or percutaneous coronary intervention, peripheral arterial disease, angioplasty or other vascular surgery, ischemic stroke, transient ischemic attack) and 151 deaths, corresponding to incidence rates of 2.1 (1.8-2.4) and 1.7 (1.4-1.9) per 100 person-years, respectively.

In models adjusted for age, gender, smoking status, hypertension, diabetes mellitus and hyperlipidemia, an increased risk for incident CVD events and all-cause mortality were associated with AUC measures of CRP and HAQ up to 2 years, and HAQ at 2 years, corresponding HRs (95% CI) for the CVD outcome: 1.04 (1.00-1.08), 1.14 (0.99-1.33), and 1.35 (1.05-1.73); and for the mortality outcome: 1.08 (1.04-1.12), 1.39 (1.19-1.63), and 1.75 (1.35-2.27), respectively. Then, RF positivity, the WBC count and the HAQ score at baseline, ESR-AUC, and DAS28-AUC were independently related to higher death rates, corresponding HRs (95% CI): 1.61 (1.13-2.31), 1.07 (1.00-1.13), 1.35 (1.04-1.75), 1.06 (1.01-1.10), and 1.09 (1.01-1.17). On the contrary, reduced CVD risk was associated with decline in the HAQ score after 1 year, and reduced mortality risk with decline in the VAS pain, HRs (95% CI) 0.75 (0.58-0.97), and 0.75 (0.58-0.97), respectively.

Conclusions and discussion: RA may worsen or hasten co-morbidity (263), and the results of the current studies also indicate that inadequate control of systemic rheumatoid inflammation during the critical first years of disease may have a negative impact on future CVD morbidity and survival. The risk of subsequent incident CVD events was inversely associated with reductions in the DAS28, HAQ score and VAS pain between inclusion and the 1-year assessment, while cumulative measures of CRP, ESR, and DAS28 over the first years of RA disease were linked to a poor prognosis, the finding that emphasizes the significance of cumulative inflammation for CVD outcomes in RA and the detrimental role of insufficient treatment of RA during the critical period after diagnosis. To date, few reports have shown relationships of changes in inflammatory and RA disease measures with CVD outcomes (264).

The present findings are in line with longitudinal inception cohort studies suggesting that the course of RA is established early, and that the most important period for therapy is the first 1–2 years. Then, baseline markers predictive for worse clinical outcomes, such as destructive joint pathology and premature mortality, might dissociate from the clinical course following appropriate treatment (34, 202). That the CVD risk in RA could be decreased if the RA disease is managed and treated with appropriate intensity, has been shown in the COBRA trial in early RA, where early intensive treatment compared with initial monotherapy improved the clinical outcomes such as survival, co-morbidities and joint damage after 11 years (374). Considering these facts, it has been suggested that RA should be controlled and monitored tight as diabetes mellitus and hypertension (265).

We also expanded earlier reports concerning the unfavourable effect of functional impairment for CVD morbidity (34, 40, 109, 150, 234, 266, 390, 398). The decline in the functional score after one year was linked to a better CVD prognosis, while the HAQ burden over the two first years implied a worse CVD outcome. At the same time, a higher HAQ score at baseline was related to a less chance of survival, but was not associated with CVD prognosis. These findings implicate that the HAQ score has differential value for prediction of outcome depending on the time-point in the course of RA disease it is measured.

Consequences of pain persistency in RA disease are not well known. Quality of life is significantly impaired in RA, and one of the most important problems is persistent pain, attributable to the combined effect of lasting synovitis, progressive joint damage and co-morbidities. Chronic pain and pain-related psychiatric illness have been shown to have a role in increased CVD related mortality in RA (95, 228). In our study, the higher was the reduction in the VAS pain between the time of diagnosis and the 1-year assessment the less was the risk for all-cause mortality, independently of demographic and traditional CVD risk factors. Although controlling pain is one of the indications of successful treatment, and it has been shown that patients given early treatment with DMARD or aggressive treatments have markedly reduced pain scores (6, 379), still, many patients with early RA continue to suffer more or less persistent pain over the course of the disease. Addressing the question of CVD risk stratification, evaluation of pain should not be neglected in clinical care of patients with RA.

The next finding in the present study was that the WBC count at RA diagnosis, but not baseline CRP or ESR measures, was independently linked to death. To our knowledge, the potential role of WBC count to predict CVD and mortality outcome has not been explored in early RA. One cross-sectional study has though documented that the WBC count, independent of age, gender and disease duration, was predictive

of mortality in patients with RA (50). In the general population, prospective studies have demonstrated an association between higher WBC count and CVD, death after acute coronary syndrome, and all-cause mortality (70, 165, 377).

Mononuclear cells (monocyte/macrophages, T-lymphocytes) play a central role in the pathogenesis of atherosclerosis by ingesting oxidized low-density lipoprotein, transforming into foam cells, and recruiting additional monocytes and macrophages from the vascular space into the subendothelial layers of large and medium-sized arteries. Infiltrates of mononuclear cells are prevalent and pathogenic within unstable coronary artery plaques (212). The proliferative activity of promonocytes can be increased by relatively mild systemic inflammatory stimuli leading to increase of monocytes count in the blood, and at the same time the characteristics of circulating monocytes also change (152). Additional studies are warranted to link elevated WBC count to increased risk for CVD and mortality in RA, and to evaluate whether WBC provides a superior predictive value to other inflammatory measures.

We recognize that the patients who volunteered for additional assessment with ultrasonography *(paper III)* might be expected to be healthier than those who did not; in fact, only one CVD event was registered before the 5-year additional assessment, which could have explained, at least partly, low rates of CVD events in that study. Also, the incidence rates in the study of a limited sample size could be influenced by chance. In the small cohort *(paper III),* rates for AMI and congestive heart failure were found at lower rates, approximately 0.1 and 0.6 per 100 person-years, compared with reported 0.5 and 2 per 100 person-years (164, 245). Underestimation of CVD rates and self-selection bias, if anything, could result in weaker associations, still, the presented results were statistically robust. Besides, our study did not have adequate power to detect small but possibly important factors, and the effect size could not be accurately estimated. Furthermore, the current results cannot be extrapolated to "pure" atherosclerotic disease as definition of cardiovascular outcomes here also encompassed outcomes with possible non-atherosclerotic underlying mechanisms, such as congestive heart failure. In the present patients we could though exclude non-ischemic causes to the heart failure such as diabetes, obesity, alcohol abuse, valvular heart disease and arrhythmia.

As to *paper IV*, the validity of the measurements was demonstrated by the reasonable incidence rates of the outcomes and distribution of several established CVD risk factors which were generally similar to those previously reported in other western inception RA populations (17, 18, 164). This study was prospective in patient enrolment and follow-up but was observational in nature and subject to limitation as uncorrected confounding, e.g. only information on lipid-lowering treatment was available rather than measures of serum lipids. We also used the composite CVD outcome, and the reported associations cannot be extrapolated for specific risks of coronary, cerebrovascular or peripheral atherosclerotic events.

4.5 IMPACT OF DISEASE-MODIFYING ANTI-RHEUMATIC DRUGS

4.5.1 Therapies and carotid atherosclerosis

Paper II. In these 114 patients with early RA, simple regression analyses showed a negative association between cumulative daily dose of methotrexate (MTX) (measured over the first five years of disease) and cIMT, p=0.038, but this association was not verified after controlling for age and gender. Neither usage of antimalarials or cumulative daily dose of glucocorticoids (GC), nor total dose and duration of therapy

with MTX or GC showed any statistically significant associations with the outcomes of carotid atherosclerosis after five years of RA disease.

Conclusions and discussion: As discussed before, the major consideration, while interpreting the results here, is a limited sample size, frequent use of concomitant glucocorticoids, a low number of patients with hypertension and diabetes at entry, inclusion of participants with pre-existing CVD, and the lack of ultrasonography examination at entry which could influence the estimates.

4.5.2 Associations of therapies with CVD and mortality

Paper III. In this study of early RA, regular use of drugs as MTX, antimalarials, biologic agents, and GC was considered if reported during at least 6 months throughout the follow-up. The use of MTX showed a protective role and was age-independently associated with a better CVD outcome, HR 0.34 ((95% CI, 0.12-0.91), hence, a risk reduction of CVD by 66% in those on MTX, while a doubled risk in those not treated with MTX. At the same time, regular use of antimalarials, biologic agents, or GC did not demonstrate any statistically significant association with the CVD outcomes.

Paper IV. In this large early RA cohort, as in *paper III*, regular use of drugs was considered if it was reported during at least 6 months throughout the follow-up. In this study, regular use of GC had an association with a poor CVD outcome, adjusted HR (95% CI) 1.65 (1.21-2.26). On the contrary, reduced CVD risk was associated with the use of any DMARD or MTX during the first year, or regular use of MTX >6 months during follow-up, corresponding adjusted HRs (95% CI): 0.63 (0.44-0.90), 0.72 (0.53-0.97), and 0.72 (0.53-0.99). Then, reduced mortality risk was independently associated with the use of biologic agents, HR 0.45 (95% CI, 0.21-0.98).

Conclusions and discussion: The findings here largely confirmed and broadened the previous reports on the protective value of anti-rheumatic treatments (23, 233), and beneficial effect of MTX use for the cardiovascular outcomes (368, 381). The fact worth considering is that the patients in these observational studies were treated in conventional care during a period before modern aggressive therapies were used. Thus, despite higher inflammatory burden measured by CRP-AUC and ESR-AUC over the first two years after disease onset, the participants with adverse outcomes had less likely been treated with MTX regularly but more likely with GC, and those who deceased during follow-up had less likely been prescribed biologic agents (*paper IV*).

The role of GC in the occurrence of CVD and increased mortality in patients with RA is more or less controversial, and both beneficial and harmful effects have been recognized (72, 137). Though confounding by indication was not accounted, the finding in *paper IV* supports the evidence linking low-dose GC therapy with adverse CVD outcomes.

Then, use of biologic agents was shown to be associated to a decrease of estimated mortality risk, a result that however cannot be considered definite owing to confounding by indication/contraindication and a low number of events in analysis. Though biological therapy is typically considered for more severe RA disease, patient selection could have produced protective effect of biologicals in the current study. However, it is worth bearing in mind the possibility of a better survival due to effective control of systemic inflammation (197). Long-term controlled trials with

randomized treatment allocation are needed in order to accurately assess the effects of biologic agents on atherogenesis and cardiovascular risk.

4.6 AGE AT ONSET OF RA AND RISK OF CVD AND MORTALITY

Paper IV. In these patients with early RA who had been followed for more than 10 years, the disease characteristics in the age groups, <65 years at RA onset vs. ≥ 65 years, were statistically similar with regard to distribution of RF positivity, the WBC count, the VAS pain and the HAQ scores at baseline, HAQ after two years of disease, and frequency of DMARD started at baseline, use of MTX and GC the first year, and regular use of GC during observation. The older participants, compared to the younger ones, had though less often ACPA antibodies, had higher baseline measures of CRP, ESR, DAS28, and were less often treated with MTX or biologic agents regularly.

Cox regression models in the age groups, adjusted for age, gender, smoking status, hypertension, diabetes and hyperlipidemia, showed that only in the younger individuals, RF or ACPA positivity, WBC count, CRP-AUC, ESR-AUC, and VAS-pain-AUC (measured over the first two years) were associated with a higher CVD risk, corresponding adjusted HRs (95% CI) 2.72 (1.48-5.02), 1.87 (1.01-3.34), 1.07 (1.01-1.14), 1.10 (1.02-1.17), and 1.06 (1.00-1.13). On the other hand, only in the elderly, reductions in CRP, ESR, and HAQ after one year, and regular use of MTX were associated with a lower risk of incident CVD event, corresponding adjusted HRs 0.94 (0.89-1.00), 0.90 (0.82-0.97), 0.71 (0.52-0.97), 0.64 (0.43-0.96), while regular use of GC heightened the risk of CVD, HR 1.69 (1.13-2.51).

As to the mortality outcome, similarly, in the younger patients, RF or ACPA positivity, WBC count at baseline, and VAS pain-AUC were associated with a higher risk of death, respective adjusted HRs (95% CI) 2.26 (1.09-4.68), 2.14 (1.05-4.35), 1.10 (1.01-1.20), and 1.09 (1.01-1.16). In the elderly patients, DAS28-AUC, and use of GC during the first year heightened the risk of death, adjusted HRs (95% CI) 1.10 (1.01-1.21), and 1.47 (1.00-2.20), respectively, while reductions in DAS28 and VAS pain after one year were associated with a lower risk of death, adjusted HRs (95% CI) 0.88 (0.78-0.99), and 0.93 (0.86-1.00), respectively.

Conclusions and discussion: The definition of early and late onset RA used in previous studies ranges from 45 years to 75 years; our distinction of age groups was pragmatic and carried out before the statistical analyses. It has been reported that in the elderly, the RA disease is characterized by a greater proportion of men than in the younger (female-to-male ratio, 1.5–2:1 versus 4–4.5:1 in the younger patients), often an insidious onset, frequent constitutional symptoms and involvement of the proximal limb joints, particularly the shoulder girdle, and remitting seronegative symmetrical synovitis. Laboratory testing may reveal a striking inflammatory response with an elevated ESR and mild anemia associated with chronic disease, but incidence of RF-positivity is low. Disease activity is usually more marked, with higher swollen and tender joint counts, a longer duration of morning stiffness, and greater alterations in quality of life indices (185, 329, 394). Thus, disease characteristics of the elderly participants in our study were for the most part typical.

To date, prognosis of RA in the elderly is unclear. Here, we explored the influence of age at RA onset on predictive models of adverse CVD outcome and mortality in early RA. Generally, in the younger patients the inflammatory burden (calculated as AUC over the first two years of disease) was shown to be associated with the study outcomes, whereas in the older patients the improvements in CRP, ESR, and DAS28

between baseline and the 1-year assessment predicted outcome. These differences between age groups might have an explanation in the fact that in the elderly inflammation is common as a consequence of many causes, the burden of which disappears in statistically analyses when adjusted for age. Still, CRP-AUC and ESR-AUC were linked to death in both age groups, but numerically the associations were attenuated in the older population with more risk factors. These results imply that different longitudinal approaches may be of importance in studies validating prognostic factors in RA. Age stratification could improve evaluation of risk factors for CVD and mortality in early RA.

The key association between age at symptom onset and clinical outcomes relates to the rate of progression of RA disease and atherosclerosis. Higher age at symptom onset is likely to be associated with a faster rate of increasing morbidity, and thus, a poor prognosis. The results of the current study, that in the older patient group the reductions in CRP, ESR, and DAS28 after one year was protective, suggest that there is a special urgency to get disease activity under control in this age group as they have less reserve to cope with inflammation and added disability.

In our analyses, association between RF, ACPA positivity and adverse outcomes was found only in the participants aged <65 years at diagnosis but not in the older patients. The inconsistency of previous reports on the role of seropositivity for prediction of clinical outcomes in RA may depend on variety of age and absolute risk of the outcome in the studied populations and the degree to which other risk factors are included in analyses. Studies in which predictive associations with the CVD or mortality outcomes were reported tend to include relatively young participants (390), while an absence of an association may reflect the older age and heavy risk factor burden of the predominantly minor cohorts (34).

Additionally, in the younger patients, similarly to measures of the inflammatory burden, VAS pain-AUC was independently associated with enhanced mortality. Then, both the HAQ score after 2 years and HAQ-AUC were predictive for mortality in both age groups. Our findings and the recent report on the lowest probability of long-term disability if persistent remission is achieved (298) highlight the concern about functional impairment also in younger patients, and the importance of treatment to target in all ages in early RA.

As to therapies, only in the elderly, regular GC use was associated with an increased risk of adverse CVD and mortality outcomes, while MTX use again showed a favorable role for CVD prognosis. Relatively limited published data are available on treatment of elderly patients with RA. Observational studies on the use of DMARDs has showed no significant difference between the older and the younger patients with regard to efficacy, side effects and discontinuation of DMARDs (67, 267), and reported minimal toxicity related to treatment (357). Thus, MTX should have a prominent role in the management of RA in older patients.

Older patients with RA, especially those with early disease, are usually responsive to treatment with low-dose GC, and the rapid dramatic improvement of symptoms experienced with such a low dose along with substantially reduced rate of joint destruction (189) shifts the risk-to-benefit ratio in favor of using steroids as first-line agents in the elderly. On the other hand, a poor CVD and survival prognosis, confirmed in the current study, should be considered. A single case-control study has previously evaluated the effect of immunosuppressive treatment (defined as a prescription for these agents within the 90 days prior to the time of the first cardiovascular event) on the risk of CVD events (hospitalization for AMI and stroke) specifically in patients with elderly onset RA, and disclosed an increased risk of CVD

events in patients on GC therapy, odds ratio (OR) for GC monotherapy: 1.5 (95% CI, 1.1–2.1), for GC combination therapy: OR 1.3 (95% CI, 0.8-2.0), and for immunosuppressive agents other than MTX with both monotherapy and combination treatment, OR 1.8 (95% CI, 1.1-3.0) (325).

4.7 GENERAL REMARKS

Although our results are intriguing in light of prior research, the potential strengths and limitations of the studies merit consideration. The principal strengths are an applicable longitudinal approach with prospective structured measurement of RA disease related factors and a broad range of biomarkers at disease onset and through the first years of disease, long duration of follow-up and comprehensive (inpatient and outpatient) data sources. All participants had a clinical diagnosis of RA made by a rheumatologist and all patients met the ACR criteria for RA. The BARFOT study database consists of typical patients treated within conventional care and seen across a variety of settings allowing a wide spectrum of RA. The BARFOT cohort more likely retained patients with milder disease, allowing generalizing from the findings of the studies. The incident RA design, in which exposure time begins at the time for diagnosis and start of systemic immunosuppressive, allows for a more valid comparison of treatments than mixing early with established disease in the same analysis. The studies emphasize that systemic inflammation can be assessed using standard routinely collected laboratory and clinical measures, providing a simple tool to monitor disease activity and, specifically, the response to treatment, which can be used as an important tool for risk stratification. The validity of our results is demonstrated by the reasonable incidence rates of the clinical outcomes and distribution of several established CVD risk factors which were generally similar to those previously reported in other western RA populations, therefore, the present findings should have wider relevance.

It is possible that the patients undergoing ultrasonography were different from the reference group of RA patients. It could be speculated that sub-clinical CVD disease might have been present already at enrolment, which might have biased our findings in favor of a positive relationship between biomarker levels and outcomes, but this confounding could have affected only estimation of a small number of adverse outcomes closer to the time of enrolment.

The major limitation is the lack of a control group and randomization to therapy. Several CVD risk factors and drug prescribing in these observational studies were influenced by the RA disease severity itself. Without random assignment of treatment, the pure impact of the inflammatory factors on the CVD and mortality risk could not be estimated, and it is difficult to infer causation between the treatments of interest and outcome risk. Further, we could not provide information about the cumulative dose of MTX and GC therapy or duration of therapy in all studies; besides, several immunosuppressive medications were commonly used in conjunction.

Although the observational nature of the current studies generally limits the conclusions of causality, nonetheless, the results provide important hypothesis-generating observations. Future large cohort studies and mechanistic studies are needed to confirm the findings.

5 PERSPECTIVES FOR THE FUTURE

Atherosclerosis and CVD are major causes of morbidity and mortality in RA. Even with the plethora of new therapies, there has not been much improvement in this risk. Although we do not fully understand what causes accelerated atherosclerosis progression, evidence strongly suggests that the complex interplay between dysfunctional immune regulation, inflammation, traditional risk factors, defective vascular repair, and therapeutics used to treat the underlying RA disease influence this process. A comprehensive biomarker panel incorporating inflammatory, RA disease and traditional CVD risk factors could be an essential tool for identifying patients at risk of accelerated atherosclerosis soon after diagnosis of RA. Clinical biomarkers predictive for poor cardiovascular outcomes are unmet needed. The clinical complexity of inflammation and accelerated atherosclerosis will probably require an integrated approach to identify and treat at-risk patients.

Atherosclerosis and RA disease are processes that take place over-time, like the effects of the risk factors assumed to influence them. The cross-sectional studies may have limited ability to estimate the temporal relationships of over-time changes of exposure variables. The potential for the future in this question is larger observational trials in inception RA cohorts incorporating longitudinal assessment of systemic inflammation and probably new imaging techniques to identify inflamed rupture-prone lesions. Along with physical examination and laboratory tests, simple qualitative patient-self reported measures should be directed more attention in research and follow-up of patients with RA. Larger, prospective, long-term, preferably randomized placebo-controlled studies are needed to elucidate the immune-inflammatory mechanisms behind progression of atherosclerotic disease in RA.

Although the world of research is drawing a clearer picture of the kind of patient who might develop CVD, it is still challenging how to determine a patient at the greater risk. For the future, the task is to identify subsets of RA patients who are at heightened risk of premature atherosclerosis based on clinical identifiers. For the present, close adherence to guidelines for primary cardiovascular prevention and a lower threshold for more aggressive interventions in RA are warranted.

6 CONCLUSIONS IN SHORT

- The increase in anti-atherogenic apolipoprotein A1 after commencement of biological therapy (anti-TNF-α and anti-CD20 agents) in RA is not agent-specific and parallel a reduction in RA disease activity, and may have a potential for at least short-term improvement in cardiovascular risk;
- The atheroprotective natural IgM antibodies against phosphorylcholine (anti-PC) increase during one year treatment of RA with TNF-α inhibitors, but decrease with the anti-CD20 therapy, which may have implications for cardiovascular risk and RA disease *per se*;
- At initiation of biologic agents to patients with RA, the low levels of anti-PC IgM antibodies predict an inferior clinical response after one year; further studies are needed to examine whether the low levels of these antibodies feature an immune-deficient state associated with promote disease activity and progression of other chronic inflammatory diseases such as atherosclerosis;
- The levels of apolipoprotein A1 over-time are negatively, while apolipoprotein B and the ratio of apolipoprotein B/apolipoprotein A1 are positively associated with carotid atherosclerosis in RA, independent of traditional cardiovascular risk factors, which suggest that apolipoproteins are independent predictors of atherosclerosis and may be useful in cardiovascular risk estimation in patients with early RA;
- In patients with RA, the bilateral carotid plaque occurrence is associated with a poor CVD prognosis, which may have implication for the clinical cardiovascular risk assessment;
- Low levels of natural anti-PC IgM are associated with the carotid plaque occurrence in RA, which extended earlier reports on atheroprotective role of these antibodies;
- Low levels of anti-PC IgM antibodies and increasing levels of oxidized LDL during the first years after RA diagnosis may link chronic inflammation in RA to an enhanced cardiovascular risk, and they might serve as indicators of a subsequent CVD event;
- In early stages of RA, the cumulative burden of inflammation and the presence of RA disease related autoantibodies have a predictive value for CVD and mortality in the younger patients, while a change in inflammatory markers may have an augmented predictive value in the older patients, which propose that age stratification could improve evaluation of risk factors for CVD and mortality in early RA;
- The progressive decline in physical function is associated with poor survival in both younger and older patients with early RA, which highlights the concern about functional impairment in all ages;
- In patients over 65 years old at RA onset, low-dose glucocorticoids may heighten CVD and mortality risks, which further supports the concern about potential long-term undesirable reactions to glucocorticoid therapy in the elderly;
- Early improvement in inflammation, pain and functional disability as well as use of methotrexate are associated with a better CVD prognosis, which emphasizes the appreciation for the need to treat RA early and more aggressively to reduce cardiovascular disease and improve long-term outcomes.

7 SVENSK SAMMANFATTNING

Reumatoid artrit (RA), så kallad ledgångsreumatism, är en kronisk inflammatorisk ledsjukdom som har påvisats vara förknippad med en minst 1.5-2-faldig riskökning för hjärt-kärlsjuklighet (CVD) och en förkortad livslängd. Ateroskleros, åderförkalkning, har liksom RA sjukdomen karaktären av en kronisk inflammatorisk process och är orsak till CVD. Uppkomst och utveckling av RA och ateroskleros har ansetts vara nära förenade. De klassiska riskfaktorer för hjärtkärlsjukdom i befolkningen, som rökning, diabetes, högt blodtryck och höga blodfetter, har inte med säkerhet samma stora betydelse hos RA patienter. Det tycks att den gemensamma inflammatoriska processen spelar en viktig roll men att även andra faktorer kan bidra. Huvudsyftet med denna avhandling var att undersöka ytterligare mekanismer bakom den förtidiga aterosklerosutvecklingen vid RA och försöka identifiera markörer som skulle kunna indikera risken att få CVD och dö.

I studierna har vi koncentrerat oss på RA sjukdomsrelaterade mått och riskfaktorer för CVD av betydelse för både inflammation och utveckling av ateroskleros. Bland dessa märks apolipoproteiner, oxiderat LDL-kolesterol (oxLDL) och skyddande naturliga antikroppar som riktas mot phosphorylcholine på oxLDL och döda kroppsceller (anti-PC). Apolipoprotein A1 (apoA1) finns i det ”goda” kolesterolet som skyddar kärlväggen och våra perifera vävnader från överskott av kolesterol. Apolipoprotein B (apoB) finns i de aterogena ”onda” lipidpartiklarna. Överskott på apoB partiklar i förhållande till apoA1 partiklar, d.v.s. en hög kvot apoB/apoA1, anses öka den aterogena belastningen på kärlväggen och risk för CVD.

På senare år har biologiska läkemedel, som t.ex. TNF-α blockerare och B-cells terapi, börjat användas i allt större utsträckning för behandling av RA. Data är inte entydiga avseende inverkan av dessa läkemedel på riskfaktorer för CVD. Vi fann att behandling med TNF-α blockerare (162 patienter med RA) eller B-cells terapi (53 patienter med RA) ledde till ökande halter av den anti-aterogena apoA1 parallellt med sjunkande sjukdomsaktivitet under ett års uppföljningstid. ApoB och den aterogena kvoten påverkades inte av behandlingarna. Dessa effekter kan vara gynnsamma och motverka utvecklingen av aterosklerosprocessen. Vidare fann vi att i TNF-α gruppen ökade halter av skyddande anti-PC medan de sjönk i B-cells gruppen. Dessa resultat talar således för potentiellt både positiva och negativa effekter avseende risk för CVD. Betydelsen av biologiska läkemedel för risk att få CVD ska följas noggrant och undersökas vidare i kommande studier.

Förutom prospektiv mätning av riskmarkörer i blodet, har 114 patienter med tidig RA undersökts med ultraljud avseende förekomst av åderförkalkning i halskärl fem år efter att diagnosen RA ställts. Vi fann att apoB, den aterogena kvoten och låga halter av anti-PC var förknippade med åderförkalkningen, medan apoA1 innebar skyddande effekter. Det kan tolkas som att apolipoproteiner och anti-PC har en viktig roll i aterosklerosutvecklingen hos RA patienter. Vidare uppföljning av samma patienter mer än 10 år efter RA sjukdomens debut visade att de patienter som hade åderförkalkningen/plaque hade högre risk att få CVD jämfört med patienter som hade negativ ultraljudsfynd, alltså kan ultraljudsundersökningen av halskärl användas som riskindikator för CVD hos patienter med RA. Vad gäller sjukdomsrelaterade faktorer var den risken att insjukna i CVD statistiskt lägre om den inflammatoriska aktiviteten, smärtan och den dagliga fysiska förmågan hade förbättrats ett år efter RA diagnosen eller om behandling med metotrexat (ett av de vanligaste läkemedlen mot inflammation vid RA) hade använts. De som löpte större hjärtkärlrisk var de patienter

som hade låga halter av anti-PC eller stigande halter av oxLDL under fem första åren efter RA diagnosen.

I en stor kohort av patienter med RA som följts sedan diagnossättandet (741 personer) mättes kliniska utfall (CVD sjukdom och dödlighet) mer än 10 år efter sjukdomsdebuten. Det visade sig att riskfaktorer skiljde sig åt beroende på åldern vid RA sjukdomens debut - före eller efter 65-årsåldern. För yngre patienter hade bördan av inflammatoriska faktorer över två första åren av RA sjukdomen och förekomsten av RA specifika autoantikroppar större prediktivt samband med kliniska utfall, medan för äldre patienter var förbättringen i inflammatoriska markörer första året närmare knuten till minskningen i antalet händelser. Liksom i den föregående studien framkom det att behandling med metotrexat hade skyddande effekter, medan användning av lågdoserade glukokortikoider var knuten till sämre klinisk prognos hos de äldre RA patienterna. Resultaten pekar alltså på att uppdelningen efter åldern vid RA sjukdomens debut kan tillföra ytterligare information för bedömning av en enskild individs hjärtkärlriskprofil. För att förbättra överlevnad vid RA tycks en tidig och effektiv behandling vara avgörande.

Riskbedömning för CVD hos patienter med RA är ett komplext beslutsbärande problem där uppföljning av sjukdomsmått, behandlingsresultat, bieffekter av terapier och biokemiska markörer ska vägas in tillsammans med de traditionella riskfaktorerna.

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—Thomas Reuen

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"In my end is my beginning."
— T. S. Eliot

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